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THE HAEMOPHILIAS: CLINICAL AND LABORATORY
INVESTIGATIONS

by

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VOLUME 1

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PREFACE

Scope of the thesis

The work for this thesis was carried out in the Regional Haemophilia Centre and the University Department of Medicine, Glasgow Royal Infirmary over the past six years. It consists of both clinical and laboratory studies into the haemorrhagic problems of patients with haemophilia and Christmas disease. In diseases with so many facets it is not possible to examine every aspect so I have focussed my attention on some outstanding clinical and laboratory problems. In the clinical section I have investigated methods of measuring the early pathological changes in the synovium of the joints, the biochemical methods of diagnosing muscle bleeding, the localisation of muscle haematomata with ultrasonography, the effect of fibrinolytic inhibitors in the reduction of bleeding after dental extraction, the incidence of and aetiology of gastro-intestinal bleeding and evaluated electroencephalography as a test of previous cerebral bleeding. In the laboratory section I look at some of the complications of therapy with plasma products, both of human and animal origin. In particular the incidence of transfusion hepatitis, the incidence of blood group antibodies, the effect of elevation of plasma fibrinogen and the effects of animal antihæmophilic factor on human blood platelets.

Some of this work has already been published and the thesis contains extracts from the following:

- 1) Christmas disease and haemophilia in Kenya
Forbes, C.D., MacKay, N., Khan, A.A.
Transactions of the Royal Society of Tropical
Medicine and Hygiene (1966), 60, 777
- 2) Cryoprecipitate therapy in haemophilia
Forbes, C.D., Hunter, J., Barr, R.D., Davidson, J.F.,
Short, D.W., McDonald, G.A., McNicol, G.P.,
Wallace, J., Douglas, A.S.
Scottish Medical Journal (1969), 14, 1
- 3) Radioactive joint scanning in haemophilia and Christmas
disease
Forbes, C.D., Greig, W.R., Prentice, C.R.M.,
McNicol, G.P.
Journal of Bone and Joint Surgery (1972), in press
- 4) Tranexamic acid in control of haemorrhage after dental
extraction in haemophilia and Christmas disease
Forbes, C.D., Barr, R.D., Reid, G., Thomson, C.,
Prentice, C.R.M., McNicol, G.P., Douglas, A.S.
British Medical Journal (1972), 1, 311
- 5) Aggregation of human platelets by commercial preparations
of bovine and porcine antihaemophilic globulin
Forbes, C.D., Barr, R.D., McNicol, G.P.,
Douglas, A.S.
Journal of Clinical Pathology (1972), 25, 210
- 6) Duplication of LDH-1 in a patient receiving multiple
transfusions
Forbes, C.D., King, I., McNicol, G.P.
Clinical Chemistry (1971), 17, 948

- 7) Rupture of the ureter due to crushing injury in a patient with severe haemophilia
Forbes, C.D., Craig, J.A., Prentice, C.R.M.,
McNicol, G.P., Levack, J.H., Ireland, J.T.,
Adams, J.F., Sutherland, G.R.
British Journal of Surgery (1971), 58, 931
- 8) Acute intravascular haemolysis associated with cephalixin therapy
Forbes, C.D., Craig, J.A., Mitchell, R.,
McNicol, G.P.
Postgraduate Medical Journal (1972), 48, 186
- 9) Aggregation of human platelets by purified porcine and bovine antihaemophilic factor
Forbes, C.D., Prentice, C.R.M.
Nature (Lond.), (1972) in press.

The detailed planning of the work, its day to day evolution and its execution were my individual responsibility, and, except where indicated in the acknowledgements, the work was entirely personally performed.

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SECTION 1

"Strange it is that our bloods
of colour, weight and heat, pour'd all together
Would quite confound distinction, yet stand off
In differences so mighty"

Alls Well That Ends Well
Act 2, Scene 3

CHAPTER 1 HAEMOPHILIA - AN OVERVIEW

CHAPTER 2 DEVELOPMENT OF KNOWLEDGE
IN BLEEDING DISORDERS

CHAPTER 1

HAEMOPHILIA - AN OVERVIEW

The word haemophilia is derived from two Greek words 'haima' - blood and 'philein' - to love and despite being senseless in the description of excess bleeding it remains in general use. In the older literature various names have been used eg. haemorrhaphilia, haematophilia, haemorrhoea, haemorrhagophilia, idiosyncrasia haemorrhagica and morbus haematicus. The first use of the word "haemophilia" is attributed to Schönlein (Virchow, 1854) but the first written use was by Hopf, a student of Schönlein, who gave a dissertation entitled "Die Hämophilie" at Würzburg in 1828.

The disease is now called haemophilia A or classic haemophilia and is due to deficient activity of antihæmophilic factor (AHF, AHG or Factor VIII). A variety has been described which is called Christmas disease in Britain but in Europe is often called haemophilia B and in the U. S. A. , plasma thromboplastin component deficiency (PTC). This is due to deficient activity of Christmas factor (CF, PTC or Factor IX).

The clinical manifestations of these two diseases are essentially similar and in this thesis no differentiation is made

between them with regard to bleeding tendency. It has been suggested that the bleeding tendency of Christmas disease is often milder than classic haemophilia (Ratnoff, 1960; McKusick, 1962(a)) but this has been disputed (Wilkinson et al., 1961; Lewis et al., 1963).

Haemophilia is often thought to be a rare or esoteric condition but this is not so. Projecting an estimate of its frequency to the current population, we would expect in Britain about 5,500 patients with classic haemophilia and about 550 with Christmas disease. In the United States there are between twenty to thirty thousand sufferers and it is probable that in all countries there are equal numbers of undiagnosed patients with mild forms of disease.

The medical and social consequences of haemophilia begin to manifest themselves in early childhood and continue throughout the lifetime of the sufferer. The high mortality in this population in their earlier years can be inferred from the survey by Katz (1970); of the 1,055 haemophiliacs surveyed, only twenty-seven men, aged sixty years and over, were found. This was 2.6 per cent of the haemophilic group compared with 20 per cent of

3.

the general population. With improvements in management and the availability of potent concentrates of plasma which can correct the clotting defect the mortality from bleeding continues to fall and it is predicted that the life expectancy of the haemophiliac may soon approach that of the general population.

The popular concept of the haemophiliac as somebody who bleeds at the slightest touch has placed him in a special category in society in which he is thought to be incapable of leading a normal existence and performing the usual social roles in the socially expected fashion. Social problems involve the patient's mother, father, brothers and sisters, and strain and distort the usual family relationships, but the person most affected is, of course, the patient in whom psychiatric symptoms and signs are well documented (Early, 1959; Agle, 1964; Mattson and Gross, 1966 (a) and (b)). From the medical point of view excess bruising may be noticed from the time the infant starts to crawl and bleeding occurs into the joints when he starts to walk. The severity of the disease dictates the number and frequency of bleeds and in this thesis the clinical classification of severity as described by Biggs and Macfarlane (1963) is used.

Severe grade: patient suffers severe recurrent joint bleeds, and muscle and subcutaneous haematomata with little or no provocation. This usually results in serious crippling.

Moderate grade: patient suffers few haemarthroses or muscle bleeds. These only occur after definite injury.

Mild grade: patient usually suffers no joint bleeds and does not bleed spontaneously. Abnormal bleeding usually only occurs after definite injury or after surgery.

These clinical grades correspond approximately to the level of AHF or CF in the plasma (Nilsson et al., 1961).

less than 1 per cent	-	severe
1 to 4 per cent	-	moderate
5 to 30 per cent	-	mild

The severe haemophiliac may suffer excess bleeding from, or into any part of the body and each site presents a different type of complication or diagnostic problem. The mild haemophiliac may not present until he has a surgical operation in middle-age; because of this, it has been suggested that the mildly affected patient now runs a greater risk than the severe patient (Ratnoff, 1963; Pappas et al., 1964).

5.

Treatment of bleeding episodes consists of replacement of the missing factor by transfusion of plasma or plasma concentrates. This may add the additional hazards of transmission of serum hepatitis or development of inhibitors.

CHAPTER 2DEVELOPMENT OF KNOWLEDGE IN
BLEEDING DISORDERS(a) Early history of bleeding disorders

It is probable that genetically determined bleeding disorders are as old as time but they seem to have been neglected by the Egyptian, Roman and Greek physicians. The earliest documented cases of a familial bleeding disorder occur in the fifth century Talmud and in Rabbinic writings thereafter. The Babylonian Talmud in the tractate Yevamoth 64b states

"For it was taught: If she circumcised her first child and he died (as a result of bleeding from the operation), and a second one also died, she must not circumcise her third child."

This decree of Rabbi Judah (redactor of the Mishneh) recognised the familial and sex-linked nature of this bleeding defect and variations of his edict have been incorporated into Rabbinic Law through the centuries and commented on by others eg. Alfasi (1013-1103), Maimonides (twelfth century).

Apart from this legal debate no cases suggestive of a bleeding disorder were described in mediaeval times except for a reference by Abul El Kassam (Albucasis), an Arabic author who lived in Cordova in the eleventh or twelfth century A.D. He described that in a certain village there were men, who, when wounded or phlebotomised, suffered an uncontrollable haemorrhage which ended only in the death of the patient.

Thereafter sporadic case reports of patients with excess bleeding may be found in the scientific literature. Philip HÜchstetter, a physician practising in Aughsburgh at the beginning of the 17th Century, speaks of a boy who haemorrhaged from the navel at birth; later in life he had repeated epistaxis, bloody stools and spontaneous ecchymosis. Banyer (1743) described the case of a 24 year old gardener from Wisbech, in the Isle of Ely, who bled profusely from a puncture wound of his foot and then nearly exsanguinated from the site of a phlebotomy performed by his physician. This boy had epistaxis, recurrent melaena and haematuria and eventually died in 1738, aged 33 years, following a trivial wound of the leg. In neither of these cases was there a documented family history.

The first probable case of haemophilia in America was recorded in an obituary in the Salem (Mass.) Gazette of March 22nd, 1791 and described the case of Isaac Zoll who died in Frederick County, Virginia, at the age of nineteen from

"a slight cut in one of his feet, with an axe. From the time of his receiving the wound, till he expired, no method could be devised to stop the bleeding."

This obituary goes on to describe the deceased's five brothers who had all died of exsanguination following minor trauma.

"one received a prick with a thorn - another, a scratch with a comb - a third, a prick with a needle - a fourth, bruised his cheek against a stove - and a fifth, received a cut in one of his thumbs."

It is of great interest that this boy's father, Henry Zoll, was twice married and only the children of the first wife were affected by this bleeding tendency.

In 1803, John C. Otto of Philadelphia described a family of bleeders by the name of Smith who had settled in the vicinity of Plymouth, New Hampshire about 1720. Otto recorded that only the males were subject to this strange affliction and the females

were exempt, although they were still capable of transmitting it to their male children. Unfortunately Otto never saw or examined the patients but learned of them from Samuel Livermore, a lawyer who represented New Hampshire in the Continental Congress. The first pedigree of haemophilia was published by Hay (1813), who followed the disease in the Appleton-Swain family through six generations spanning 172 years and containing twenty haemophilic males. This family has been reinvestigated by several authors since then (Osler, 1885; McKusick, 1962 (b)) and has now been traced over four hundred years and thirteen generations. The disease was identified originally in a male born in Bristol, England, in 1630 who came to Newbury, Massachusetts in 1639.

The first European notices of the disease are case reports by the Editor of *Sammlung auserlesener Abhandlungen* (1805) and by Consbruck (1810), followed by careful clinical and post-mortem observations by Blagden (1817) and Wilson (1819). It is of interest that Meckel (1816) postulated a coagulation defect in haemophilic blood but his suggestion was ignored for eighty years.

The next publication marks the transition from isolated case reports to an attempt at scientific description of the disease.

In 1820, Nasse published a collection of the cases available at that time and gave a general review of the disease. He was of the opinion that the blood was of "unusual fluidity" and this was due to "hyperoxidation". From study of the family histories came "Nasse's Law" which stated that haemophilia occurs only in males and is transmitted by unaffected females.

After 1820, the history of haemophilia is almost totally contained in the German literature and between 1830 and 1860 complete descriptions of the disease are met within the text books of Schönlein, Canstatt, Neumann, and Fuchs. At this time there was no real clue to the aetiology of the disease but it was thought by Schönlein to be due to "cyanosis and malformation of the heart" and by others to be due to "anomalous gout" or "scrofula", this despite the adequate post-mortem examinations reported by Virchow (1854).

Grandidier (1855) collected all cases published up to that time of abnormal bleeding and found that there were 452 males and 32 females. These numbers are difficult to evaluate as they contain cases of umbilical haemorrhage, menorrhagia and haemorrhage after circumcision as well as cases of haemorrhagic disease suggestive of haemophilia.

In 1872 Legg wrote an excellent 'Treatise on Haemophilia' describing the genetic transmission of the disease,

"The daughters possess in a very high degree the faculty of transmitting haemophilia to their sons. The women may appear perfectly healthy; they may marry perfectly healthy husbands; and yet bear a family, all the boys of which shall be bleeders."

He also described in detail the bleeding problems associated with the disease, the causes of mortality, the psychiatric aspects, the episodic bleeding and the current treatment. All this seems to have been neglected till it was rediscovered by later authors.

In 1893 Wright discovered that the clotting time of the patient's blood was prolonged and in 1911 Addis and Minot and Lee (1916) observed that the addition of normal blood could correct the clotting defect in the haemophilic blood.

In their classical monograph Bulloch and Fildes (1912) surveyed the disease in 235 patients and their relatives. They re-emphasised the limitation of the disease to males but gave no credence to earlier descriptions of haemophilia in the female despite case reports strongly suggestive of a bleeding tendency. Indeed the female patient described by Treves in 1886 has subsequently been shown to belong to a family afflicted with classic

haemophilia (Merskey, 1951).

In the first few years of this century the diagnosis of haemophilia depended on the clinical and family history plus the finding of prolongation of the whole blood clotting time (Liston, 1839; Wright, 1893) in patients with normal levels of prothrombin (Howell and Cekada, 1926).

Treatment of the bleeding episodes up to this point varied from the bizarre to the lethal despite the demonstration by Lane (1840) of the beneficial value of blood transfusion. This was rediscovered by Weil (1906) and Minot and Lee in the U. S. A. (1916). The use of plasma was then shown to be superior to whole blood by Feissly (1924) and Payne and Steen (1929) and this treatment became standard practice for thirty years.

The first major biochemical advance occurred in 1935 when Bendien and van Creveld, then Patek and Taylor (1937) found that platelet free plasma, precipitated with water at a pH of 5.3 - 5.8, yielded a substance which was capable of correcting the clotting abnormality. This was confirmed by Lewis et al. (1946) and Brinkhous (1947) and the protein further characterised.

Until 1947, haemophilia was considered to be a single disease entity, then Pavlovsky observed that blood from one haemophiliac

could correct the clotting defect in another. This phenomenon was demonstrable both in vivo and in vitro (Schulman and Smith, 1952; Koller et al., 1950). Coincidental with these clinical findings the development of tests of thromboplastin generation (Biggs and Douglas, 1953) confirmed that there were two separate groups of patients. This observation was confirmed clinically by the finding of a group of "haemophilic" patients whose defect could be corrected by the infusion of haemophilic blood plasma (Biggs et al., 1952; Aggeler et al., 1952). This new disease was called Christmas disease and is associated with lack of a factor found in serum called Christmas factor (C. F., factor IX or plasma thromboplastin component (P. T. C.)).

As knowledge increased and new clotting tests were devised it became apparent that there were other bleeding disorders besides the haemophilias. In 1947, a clotting deficiency was described by Owren in a patient with normal prothrombin and fibrinogen levels. This plasma would not clot readily with tissue extract and calcium ions and the defect was called Factor V deficiency. In the course of time it became apparent that this factor was identical with the "labile" factor described by Quick (1943) and to the accelerator globulin (Ac-globulin) of Ware and Seegers (1948).

Further investigations have shown the need for two additional factors participating in clotting with tissue thromboplastin, Factor VII (De Vries et al., 1949; Alexander et al., 1951) and Factor X (Telfer et al., 1956; Hougie et al., 1957). All these defects present with bleeding which is clinically indistinguishable from that of haemophilia and it is probable that early case records included patients with these defects.

As the pathways of blood coagulation have become better understood it is apparent that defects can be found in the laboratory which are not manifested clinically eg. Hageman factor deficiency (Ratnoff and Colopy, 1955) is not usually associated with bleeding and deficiency of plasma thromboplastin antecedent (Rosenthal et al., 1953) is associated with bleeding in only about two-thirds of cases. Deficiency of fibrinogen may be associated with only a mild bleeding defect.

(b) Distribution of haemophilia and Christmas disease

Classical haemophilia probably exists in about 3 - 4 per 100,000 of the population in Britain (Biggs and Macfarlane, 1966). A similar figure is found in Europe (Hardisty and Ingram, 1965) and in the United States (Ratnoff, 1960; Lewis et al., 1963). It

is important to remember the dictum of Legg (1872).

".... the number of cases in a given country depends upon the previous education of the medical men, and the interest which they take in the disease."

He points out that in 1872 Germany had 50 per cent of cases, England less than 20 per cent, France, U.S.A. and Switzerland 10 per cent each.

The disease has now been described in every racial group (Prentice and Ratnoff, 1967) but is said to be uncommon in the American Negro (Lewis et al., 1963), the Japanese (Yoshida, 1961) and the Bantu (Merskey, 1958). This last statement is probably correct and may apply to other negroid stock. In a recent study of East African negroes from various Kenyan tribes we found only three families with Christmas disease and five families with haemophilia (Forbes et al., 1966). This observation has been confirmed by other investigators working in Uganda (Lothe, 1968). As laboratory facilities are extremely deficient in both these countries it is difficult to draw definite conclusions but if the incidence of these diseases was similar to that in Europe and America one would have expected about 270 patients in Kenya alone. It is

possible that the number of adult haemophiliacs is in fact low due to the high mortality which would be expected following ritual circumcision carried out at puberty.

There are also wide variations in the incidence of bleeders within certain countries, eg. in the Tenna Valley in Switzerland (Moor-Jankowski et al., 1957) and in the Amish communities in Ohio and Pennsylvania (Wall et al., 1964) the incidence of Christmas disease is high due to inbreeding.

(c) The inheritance of haemophilia and Christmas disease

Only about two-thirds of patients with these disorders have a family history of excess bleeding (Aggeler et al., 1961; Schulman, 1962; Macfarlane, 1962). The interpretation of this figure is difficult as few people know the medical history of their forebears beyond two generations and many of the cases could have been transmitted through successive generations of female carriers. When extremely active attempts are made to trace back family histories, a higher incidence (80 per cent) of positive histories is found (Ramgren, 1962 (a); Quick, 1966). Occasionally the patient's disorder must be due to a fresh gene mutation.

Genetic defect in haemophilia

The formation of defective molecules of antihaemophilic factor is probably due to the presence of an abnormal recessive allele at the gene locus on the X chromosome controlling synthesis of this factor. This locus is close to those determining deutan colour blindness (Whittaker et al., 1962) and glucose 6-phosphate dehydrogenase synthesis (McKusick, 1962 (a)). Males, possessing this one abnormal X chromosome produce a protein, antigenically similar and in an equal amount to normal AHF but with deficient activity (Denson et al., 1968; Hoyer and Breckenridge, 1968; Bennet et al., 1970; Zimmerman and Ratnoff, 1971 (a)). Females possessing an additional normal X chromosome are thought to produce twice as much protein antigenically similar to normal AHF but with an activity of half that which one might expect (Zimmerman and Ratnoff, 1971 (b)). The suggestion of Zimmerman and Ratnoff is that the abnormal X chromosome produces an abnormal protein with no activity and the normal X chromosome produces protein with activity. They suggest that this will provide a method of identification of carrier females. The sons of a haemophilic male do not inherit his X chromosome and are unaffected, but all

the haemophilic daughters will be carriers, since they inherit the affected X chromosome. In the rare instance of marriage between a haemophilic male and a carrier female, the daughters have an even chance of being haemophilic, inheriting the abnormal allele from each parent. At least four authentic cases of haemophilia in the human female have been reported (Israels et al., 1951; Merskey, 1951), but examination of the chromosomes of one such female showed her to be genetically male (Nilsson et al., 1959). In canine haemophilia the mating of a haemophilic dog to a carrier female results in the birth of haemophilic females (Brinkhous and Graham, 1950).

Genetic defect in Christmas disease

The loci for Christmas factor and haemophilia are apparently far apart on the X chromosome, and only a linkage with the newly described serum protein designated as X_m has been found (Berg and Bearn, 1966). It is thought that in Christmas disease there are two varieties, one in which there seems to be a complete absence of protein and another, much rarer type in which the plasma contains a factor antigenically indistinguishable from normal Christmas factor (Hougie and Twomey, 1967).

The concentration of Christmas factor in the plasma of females who are heterozygous carriers of Christmas disease is

about half that of normal individuals (Barrow et al., 1960; Bolton and Clark, 1959; Simpson and Biggs, 1962). These carriers are usually asymptomatic but in a few cases the concentration of Christmas factor is so low that a bleeding tendency exists (Hardisty, 1957; Nillehn and Nilsson, 1962).

Cases have been recorded in which classic haemophilia and Christmas disease are thought to co-exist (Soulier and Larrieu, 1953; Hill and Speer, 1955; Graham, 1957; Nilsson et al., 1961). Diseases with similar features to haemophilia and Christmas disease have been described in dogs (Graham et al., 1949; Mustard et al., 1960) and in a horse (Nossel et al., 1962).

(d) Current thoughts on the role of AHF and Christmas factor in blood coagulation

The end stage of the coagulation process is the production of an insoluble fibrin clot. Numerous theories to explain the preceding series of reactions have been proposed (Davie and Ratnoff, 1965; Seegers, 1965; Quick, 1966 (a)). In 1964, two essentially identical systems termed a "waterfall" and a "cascade" were suggested to explain the enzymatic nature of blood clotting (Davie and Ratnoff, 1964; Macfarlane, 1964). In these systems inactive protein precursors in the plasma were activated in a stepwise manner, one protein acting as an enzyme the other as a substrate. A current theoretical concept is shown in fig. 1.

In the presence of activated plasma thromboplastin antecedent (PTA) and divalent calcium ions, Christmas factor is converted to an activated form (Waalder, 1959; Ratnoff and Davie, 1962; Nossel, 1964). Activation involves minor proteolysis as there is a change in electrophoretic mobility of Christmas factor on starch block electrophoresis (Schiffman et al., 1964). Activated PTA may function as an enzyme by virtue of its esterase activity (Kingdon et al., 1964). Recent work, using purified factors, suggests that AHF is not activated but forms a complex with CF (a), phospholipid and calcium ions (Hougie et al., 1967; Barton, 1967). It is thought that this complex then activates the Stuart-Prower factor (Factor X).

It is probable that there exists a feed back activation of AHF by thrombin (Rapaport et al., 1963 and 1965) and it has been shown that thrombin-modified AHF formed factor X activator at a rate nearly twenty times faster than a control sample but the final amount of factor X activator was unaltered (Lundblad et al., 1971).

These schemes of coagulation remain the essential theoretical basis of blood coagulation despite our clinical knowledge that patients with Hageman factor deficiency rarely bleed excessively (Ratnoff and Colopy, 1955).

SECTION 2

CLINICAL AND LABORATORY INVESTIGATIONS

"If you prick us, do we not bleed?"

The Merchant of Venice,
Act III, Scene 1

- CHAPTER 3 JOINT DISEASE IN HAEMOPHILIA
- CHAPTER 4 MUSCLE BLEEDING IN HAEMOPHILIA
- CHAPTER 5 DENTAL EXTRACTION IN HAEMOPHILIA
- CHAPTER 6 GASTROINTESTINAL BLEEDING IN
HAEMOPHILIA
- CHAPTER 7 ELECTROENCEPHALOGRAPHY IN
HAEMOPHILIA

CHAPTER 3INVESTIGATION OF JOINT CHANGES IN HAEMOPHILIA(a) Introduction

The clinical features of acute haemarthrosis in haemophilia were well described in early clinical case reports (Davis, 1826) but it was not initially appreciated that the acutely swollen joint contained blood. This was first suggested by Dubois (1838) but the suggestion was met with great resistance until Reinert (1869) described a patient with a bleeding tendency who developed an acute swelling of the shoulder joint which 'suppurated' and liberated a great quantity of blood. This observation was confirmed by Assman (1869) when he punctured the knee joint of a haemophilic with an acute swelling and allowed a considerable quantity of fluid blood to escape. The earliest post mortem examination of the chronic joint changes in haemophilia was that of Lemp (1857) who described pigmentation and hypertrophy of the synovium associated with dissolution of the articular cartilage. Prior to this J. D. Felt (1834) a local New England (non-medical) historian, wrote about the Appleton-Swain family (vide supra) and stated that these bleeders were "subject to severe and premature rheumatism". König (1892) published a detailed account of the pathological changes in a large

series of haemophilic joints and suggested that this resulted from recurrent bleeding into the joint space. Histology of both the acute and chronic joints changes has now been well documented by numerous investigators (Freund, 1925; Key, 1932; Collins, 1949; De Palma and Cotler, 1956; Marion, 1965) and observations have also been made in haemophilic dogs with joint disease (Swanton, 1957 and 1959).

In experimental studies, injection of whole blood into the joints of animals produced an inflammatory response with hypertrophy of the synovial villi (Key, 1929; Young and Hudacek, 1954; Trueta, 1966). The effect of a single injection was short lived but more pronounced effects were found with multiple injections which produced histological appearances similar to those found in human haemophilic joints (Key, 1932). It is probable that in haemophilia a vicious circle is set up in which crushing of the hypertrophied villi occurs as a result of normal joint movements and this leads to further joint bleeding, which in turn leads to hypertrophy and hyperaemia of the synovium. It has been suggested that prophylactic therapy with plasma or plasma concentrates may be of value in preventing acute joint bleeding and so might break this

vicious circle (Ahlberg, 1965; Kaspar et al., 1970; Brinkhous et al., 1970; Hirschman et al., 1970; van Creveld, 1971).

However, there is some evidence that deterioration continues, as judged radiologically, if prophylactic therapy is attempted when bone disease is established (van Creveld et al., 1971).

Joint disease is probably the greatest single cause of morbidity in haemophilia. After repeated bleeding episodes the joints become disorganised with obvious external deformity, loss of function, pain and limitation of movement. The adjacent muscle cuff undergoes atrophy and support of the joint is further weakened. In the severely affected haemophilic child it has been shown that by the age of ten years, eighty per cent have chronic arthritis of the knee joints (Kerr, 1963).

Prevention of this chronic joint process would therefore seem to be important in the overall management of haemophilia. Despite many approaches, such as the use of plasma and plasma concentrates, fibrinolytic inhibitors and steroids there is little evidence that the basic pathological process is substantially altered, although there are dramatic clinical changes in the signs and symptoms of the acute joint when plasma products are infused in adequate amounts.

Proper assessment of the effects of new therapeutic agents is difficult due to the lack of objective tests which can be easily repeated and which reflect changes within the joint over a period of time. Radiology is unfortunately of little value in the detection of early changes in the soft tissues of the joint. By the time cartilage and bone changes are apparent the arthritic process is probably irreversible (van Creveld et al., 1971) and the radiological changes which do occur (Jordan, 1958; De Palma, 1967) probably reflect some long forgotten bleed into the joint.

In this chapter is described an attempt to quantitate the early joint abnormalities in haemophilia and Christmas disease using radioactive joint scanning and thermography. A clinical scoring system has also been devised to compare with these objective tests.

(b) Radioactive joint scans

Introduction

Intravenous technetium (^{99m}Tc), half-life six hours, is an ideal isotope for joint scanning and has been used to study other joint diseases (Anon, 1968). The radiation dose to the body of 0.1 rads is extremely small (Belcher and Vetter, 1971). The scans, which consist of a pictorial image of isotope localisation in and

around the joint, show changes in regional blood flow at the time of study and are abnormal during acute inflammation (McCarty et al., 1970 (a); Weiss et al., 1965; Weiss et al., 1966; Whaley et al., 1968). While visualisation of the isotope in an inflamed joint reflects, mainly, increased vascularity of the synovial membrane and other joint tissues (Alarçon-Segovia et al., 1967; Whaley et al., 1968), part of the localisation of the radioactivity may be due directly to the synovium binding the technetium (Green and Hays, 1969; McCarty et al., 1970 (b); Mowat et al., 1971).

For these reasons joint scans are abnormal in a variety of inflammatory joint diseases, such as rheumatoid arthritis, gout, psoriatic arthritis, acute pseudo-gout and experimental synovitis, but are not characteristically specific for one type of inflammatory change. Nevertheless, as McCarty et al. (1970 (b)) have shown, the joint scan may be much more abnormal than the degree of symptoms and signs would suggest and since they can be repeated at intervals and do give an index of blood flow in a joint it was thought this technique might be helpful as a measure of the extent

of the synovial lesion in the joints of haemophiliacs. The present study was confined to the knee joints where scanning is optimum for clarity and comparative work.

Method

Five millicuries of technetium (^{99m}Tc) as sodium pertechnetate (NaTcO_4) were given as a single bolus intravenously to each patient and the knee joint scans commenced 25 minutes later as suggested by Whalley et al., (1968). The image of the radioactivity distribution in the joint was obtained using a rectilinear scintiscanner (Selo Model DS4/4) which has two 5 inch detectors. Scans were performed of both knees first in the antero-posterior and then in the lateral positions. The display was in colour; line spacing and scan speed being adjusted for each patient, joint, and position. The time taken to scan the knees of each person was approximately 15 minutes.

Various classification approaches were considered but it was ultimately decided that the most practical method of categorisation of the images should be simple and visual. Accordingly the scans from each knee joint were examined and then said to be normal, if they were obviously similar to knee joint scans from controls in other studies (Group 0). In the normal knee there is very little

isotope uptake. If the scans were obviously abnormal in width, depth and degree of isotope uptake, with or without irregular localisation they were said to be group 3. This group showed a very high isotope uptake. Scans which were obviously abnormal but not grossly so were given grade 2. Slightly abnormal scans were grade 1 (see figs. 2, 3, 4, 5).

Thirty-five patients were included in this study, ranging in age from 11 - 60 years, and informed consent was obtained from all subjects or parents. Twenty-eight patients had classical haemophilia and seven had Christmas disease. There was no clinical evidence of any other variety of joint disease and the anti-nuclear factor and Rose-Waaler tests were negative.

Classification of the clinical severity of haemophilia was described by Biggs and Macfarlane (1963). Patients were graded as 'severe' if they had suffered repeated haemarthroses with serious crippling and deep tissue haemorrhages with little or no provocation, as 'moderate' if they had sustained few haemarthroses with no serious crippling or occasional muscle haematomas and 'mild' if they had no haemarthrosis or other spontaneous bleeding, and merely gave a history of abnormal bleeding after definite injury. 18 of our

patients were 'severe', 6 were 'moderate' and 11 were 'mild'.

Each of the following clinical features were arbitrarily graded from 0 - 3; these features were pain, effusion, temperature, crepitus and degree of contracture. This we have called the 'signs score'. The number of previous bleeding episodes into the knee joints were also graded from 0 - 3, a score of 0 being no previous haemarthrosis, 1 being one haemarthrosis, 2 being less than one haemarthrosis per year and 3 being more than one haemarthrosis per year; this we have called the 'incidence score'.

Six patients were seen with acute haemarthroses on a background of previous episodes of joint bleeding and 15 with chronic joint disease of varying degrees of severity. Two patients had subacute disease at the time of examination. This category was defined as a swollen, non-tender joint with a normal range of movement, in which there was no history of an acute bleed in the previous six months. 12 patients gave no previous history of joint bleeds and had normal knee joints on clinical examination. In 6 of the patients clinical examination and scanning was repeated.

Results

Acute haemarthrosis (Table 1) Six patients (1 - 6) aged from 14 - 60 years were studied in this group. All had a severe defect and had sustained frequent bleeding episodes into both knees. Every

joint had an incidence of bleeding score of 3. The 'signs' score reflected the clinical changes at the time of examination and in the acutely affected joint these scores ranged from 8 - 11. The other knee joint in all patients had a low score (0 - 4). This was probably the result of chronic changes in the joint. Radio-active joint scans in all the acutely affected joints were grossly abnormal (grade 3).

All patients in this group were energetically treated with plasma concentrate (cryoprecipitate or Christmas factor concentrate) with rapid resolution of the clinical signs and a fall in the signs score which usually reached the baseline for the joint in 48 - 72 hours.

Two of the patients (4, 5) have had serial joint scans following the acute episode. The scans remained abnormal (grade 3) for at least four weeks and in one patient the scan was still grade 2 at twelve weeks. Unfortunately he sustained another acute haemarthrosis in the same joint and the signs score rose dramatically and the scan became grossly abnormal (Table 2).

Two patients (2, 3) with chronic joint disease were seen at a later time after they developed acute bleeding into a knee joint.

Table 3 shows the changes in the 'signs' score and in the scans in these patients.

Lateral scanning of the knees failed to localise the isotope to any particular part of the joint.

In acute joint disease there is a good correlation between the 'signs' score and the abnormality of the scan (fig. 6).

Subacute joint disease

Both patients (7, 8) in this group had painless swelling of one joint which had been present for at least eight weeks. There had been no history suggestive of an acute bleeding episode in the previous six months. One patient was a moderately affected haemophiliac and the other had severe Christmas disease. The results are shown in Table 4. In both patients the affected joint had an abnormal incidence of bleeding score (grade 3) and the 'signs' score was elevated although not so abnormal as in the acute joint series.

Both patients received adequate haemostatic doses of plasma products and clinical resolution of the joint swelling took place over the period of a few days. The 'signs' score fell in patient 7 from 0 : 9 to 0 : 1 and in patient 8 from 6 : 1 to 1 : 1. In patient 8 the scan was repeated at 6 weeks and showed a change

from grade 3 to grade 1. This may represent a baseline for this joint as the patient has chronic arthropathy as judged radiologically.

Chronic joint disease

The details of the fifteen patients (9 - 23) are shown in Table 5. Four had a moderate defect and eleven were severely affected. Two patients (18, 23) had Christmas disease and the rest had haemophilia.

All patients had an abnormal 'signs' score and abnormal scan in one or both knees. As shown in figure 7 there is a poor correlation between the clinical signs and the radioactive scan. There are joints in which the scan is grade 2 or 3 and in which there was a zero or low 'sign' score (Patient 9, 11, 12, 13, 23). Similarly there seemed to be no good correlation between the scans and the incidence of bleeding score, there being patients with no history of bleeding into one joint who had abnormal scans (patients 11, 23) and patients with a history of bleeding into a joint who had normal scans (patient 21).

No detectable joint disease

The details of the twelve patients (24 - 35) with no detectable joint disease are shown in Table 6. They were aged from 11 - 52 years.

Eleven of the twelve patients had a mild degree of haemophilia. One patient (31) with a moderate degree had been afflicted with severe paralytic polio as a child and had never borne weight on the joints of the lower limbs; however, he had sustained multiple other bleeding problems consistent with this degree of defect. As can be seen none of these patients had sustained bleeding into the joints and none had any clinical joint abnormality. However, four of the patients had bilateral abnormal scans (grade 2 or 3).

(c) Thermography

Introduction

Thermography is the pictorial representation of infra-red emission from the body surface and has been used before in the investigation of inflammatory joint diseases such as rheumatoid arthritis (Boos, 1964; Cosh, 1966; Cosh and Ring, 1967; Haberman et al., 1968; Goldie, 1969; Viitanen and Laaksonen, 1970), Tietze's disease (Aarts, 1969), spondylitis (Agarwal et al., 1970), Osgood-Schlatters disease and osteomyelitis (Aarts, 1969) and simple 'sprains' (Albert et al., 1964).

In inflammatory joint disease the surface pattern of raised skin temperature can be easily demonstrated. In the knee joint the skin temperature over the patella is thought to be an approximate guide to intra-articular temperature although 2 - 3°C lower (Lloyd-Williams, 1969). It was felt that thermography might be helpful in investigation of haemophilic joints to measure the extent of increased synovial vascularity. We have also compared the results of the thermographs taken in acute and chronic haemophilic joint disease with the number of previous bleeding episodes and the clinical assessment of the joint abnormality, and have compared this method with radioactive knee joint scanning using technetium.

Methods and patients studied

Fourteen patients with haemophilia or Christmas disease were studied. Their ages ranged from ten to sixty years, thirteen had haemophilia and one had Christmas disease. There was no clinical or laboratory evidence of any other type of joint disease being present in the series and the Rose-Waaler and anti-nuclear factor tests were negative. Five patients had acute haemarthroses on a background of chronic joint disease, six had chronic joint disease

of various severity with no recent episodes of acute haemarthrosis and three patient with mild haemophilia had no apparent joint disease.

The equipment used for thermography was the Bofors I.R. camera and display unit. This has an indium-antimonide detector cooled by liquid nitrogen; it will distinguish temperature variations of the order of 0.2°C and has the facility for demonstrating isothermal bands.

The thermographic examinations were carried out with the subject sitting in a chair with the knees several inches apart and flexed approximately 30° in order to provide a standard position which could be adopted by the arthritic patients. The temperature of the room was kept as constant as possible (21°C to 24°C). Lloyd-Williams et al. (1961) recommended a constant level of less than 20°C . In order to achieve equilibrium before starting the recording, the unclothed lower limbs were exposed for a period of 15 minutes whilst a fan was directed towards them. Photographs of the thermographic pattern and of a series of isotherms as displayed on the oscilloscope were taken with an attached 35 mm. camera,

according to a technique previously described by Feasey et al. (1970). This provided a permanent record of the examination.

The 35 mm. thermograph records were examined by an observer experienced in this technique and from observations of the films and the isotherms a crude scoring system was devised. Thermographs that were normal were given a score of 0, if slightly abnormal a score of 1, markedly abnormal a score of 2 and for grossly abnormal a score of 3. Examples of such thermograms are shown in figs. 8, 9, 10, 11. An example of the use of isotherms is shown in fig. 12.

Results

The results of thermography in the three groups of patients are shown in fig. 13 and Table 7.

Acute haemarthrosis

Each of the five patients in this group was severely affected and in each instance the thermograph was grossly abnormal (score 3) in the joint with the acute haemarthrosis. In no joint was there any localised area of heat emission. By use of isotherms it is possible to measure the temperature difference between the affected and unaffected joint. Figs. 11, 12 show a rise in temperature of

5°C in a joint with an acute haemarthrosis. There is a good correlation between the abnormality of the thermograph and the signs score. There is no correlation with the number of previous bleeding episodes.

Chronic joint disease

The details of these six patients are shown in Table 7.

All six had a severe degree of haemophilia and had sustained frequent bleeds into both knee joints. All six had abnormal thermographs of one or other knee joint but the abnormality of the thermographs did not correspond well with either the number of previous bleeding episodes of the 'signs' score, there being patients with grossly abnormal joints on clinical examination with only a slight abnormality on the thermograph (patient 9).

No detectable joint disease

Three patients with no detectable joint disease who had mild haemophilia were also examined. All three had abnormal thermographs (score 1). There was no history of previous bleeding into the joints in any patient. These joints were radiologically normal.

Comparison of thermography and radioactive scans

In acute joint disease (fig. 14) there is a crude positive correlation between the scans and thermographs, all are abnormal. The five scans are grade 3 and four of the five thermographs are grade 3, the other being grade 2.

In chronic joint disease (fig. 15) there is poor correlation between the scans and the thermographs with wide discrepancies in individual joints eg. some joints appearing to have a normal thermograph and others having a grade 3 scan. The scans are more often abnormal than the thermograph.

The numbers in the third group are too small for comment.

(d) Discussion

One of the earliest, most frequent and most disabling manifestations of haemophilia is acute haemarthrosis. Symptoms of joint bleeding may occur as soon as the child starts to walk (Ramgren, 1962 (b)) and by the age of ten years, eighty per cent of severe haemophiliacs have abnormal knee joints (Kerr, 1963).

There is, as yet, no satisfactory objective method of measuring the changes which occur in the haemophilic joint over a period of time. Obviously clinical examination can give a crude

assessment of pain and limitation of function and similarly radiology of the joint shows changes which may have results from some long forgotten haemarthrosis.

In this present study we have used two new techniques in the investigation of haemophilic joint disease. From work carried out in rheumatoid arthritis it has been shown that the radioactive scans reflect blood flow in the synovium (Weiss et al., 1965). Abnormality of the scans indicates hyperaemia of the synovium and other joint tissues. Thermography provides a picture of heat emission from the joint and this is also thought to reflect synovial blood flow (Goldie, 1969).

The aetiology of the signs of inflammation in the joint in acute haemophilic haemarthrosis is not known but it has been suggested that pain is partly due to capsular distension and that intra-articular bleeding will continue until the intra-synovial pressure exceeds that of the capillaries and arterioles of the bleeding site. The signs of inflammation may also be associated with the generation of kinin-like material from components in the extravasated blood (Ratnoff, 1966; Moskowitz et al., 1970).

In acute haemarthrosis the clinical signs can be correlated with the degree of abnormality of the radioactive scans and with the thermographs, the abnormalities of which reflect changes in the synovial blood flow. This hyperaemia of the synovium can be seen histologically in the joints of haemophilic patients after bleeding (Key, 1932) and in experimental animals after injection of blood (Key, 1929; Young and Hudacek, 1954; Trueta, 1966; Roy and Ghadially, 1966). The histological abnormality in experimental animals is short lived after a single injection of blood but more pronounced effects can be produced by multiple injections of blood. After a single injection the synovium may return to normal but permanent changes are produced by multiple injections. The slow resolution of the synovial changes after acute bleeding is well shown in our patients in which the scans took up to three months to return to the baseline, indicating that temporary synovial hypertrophy is partly involved in the pathological process.

The subacute classification represents a variation of the processes seen in the acute joint. In both our patients there had been no clinical evidence of recent acute bleeding and the joints had been swollen, hot and painless for over eight weeks. However, the scans were identical with those seen in acute bleeding. Repeat

scanning in patient 8 six weeks later showed only slight improvement despite total clinical resolution with adequate haemostatic therapy.

All the patients with chronic joint disease had abnormal scans and thermographs of one or both knees. None of the patients in this group had a history of recent acute haemarthrosis so the abnormal scans presumably represent a chronic state in which there is increased vascularity in the synovium. This is in keeping with many previous descriptions of the histology of these chronic joints (Bokelman, 1881; Konig, 1892; Freund, 1925; Collins, 1949; Rodnan et al., 1959; De Palma and Cotler, 1956; Marion, 1965; Trueta, 1966), in which the synovium was found to be markedly thickened, hyperplastic and endowed richly with vascular channels. Storti (1969) has described a hypertrophic-angiomatous type of synovium with thin-walled varicose veins on the surface which bled even when touched with a gauze swab.

In the group of patients with no clinically detectable joint disease and no previous history of bleeding into the knee joints, four patients had bilateral abnormal scans and three had abnormal thermographs. The abnormalities presumably are a reflection of

subclinical bleeding into the joints, a condition which is analogous to the asymptomatic bleeding from the renal capillaries which we have previously described in mild haemophilia (Prentice et al., 1971).

When the two methods are compared there is agreement only in the acute haemarthrosis. Both methods are more sensitive than clinical examination and this confirms the findings of Cosh et al.(1970) and Maxfield et al. (1972). These authors suggest that thermography shows a more superficial pattern, while the scans demonstrate the deep synovial area.

Recently synovectomy has been carried out in patients with chronic haemophilic joint disease (Storti et al., 1969, 1970). The preliminary results seem encouraging but as yet no long term follow up is available. These two objective tests should prove of value in the selection of patients for this operation and in the assessment of the short and long term results of the synovectomy. They may also prove of value in the assessment of steroids and antifibrinolytic agents in the treatment of acute joint bleeds.

CHAPTER 4MUSCLE BLEEDING IN HAEMOPHILIA(a) Introduction

Bleeding into muscles has been recognised from early times as a frequent cause of morbidity in haemophilia. Usually this occurs after trauma (Lafargue, 1835) but can occur spontaneously or after emotional stress (Fournier, 1851). The swelling may reach enormous proportions (Vieli, 1863) and has caused death, on occasion, from exsanguination. It was not until 1839 that Hopf, on opening one of these swellings with a caustic, showed that they contained altered blood; the result on this occasion for the patient was exsanguination from the wound and death. In two modern surveys muscle haematomata were shown to be the third commonest type of bleeding, the incidence varying from 65 - 82 per cent (Hartman and Diamond, 1957; Wilkinson et al., 1961).

Large muscle haematomata may produce compression of adjacent nerves, blood vessels or other structures. In the muscle itself there is large scale destruction of muscle fibres, partly as a

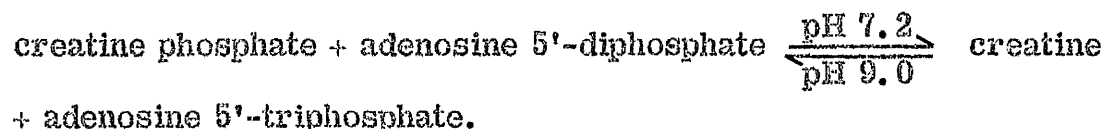
result of mechanical disruption and partly due to ischaemia, as the venous circulation, then the arterial supply is interrupted by the rising pressure within the haematoma. It is thought that, as the healing process proceeds, muscle fibres are replaced by fibrous tissue, which may produce contractures, particularly in the calf and forearm muscles (Trueta, 1966). A rather unusual feature of an untreated muscle haematoma is the development of a chronic haematoma (pseudotumour) which may grow to large proportions and produce erosion of adjacent bones and other tissues.

Muscle haematomata do not usually present a diagnostic problem as they often develop in a peripheral situation but if they occur within the abdomen the diagnosis is more difficult. In the first section of this chapter we describe changes in serum enzyme activities after muscle bleeding. This we believe can be used as the basis of a test to prove the presence of intramuscular bleeding. In the second section the use of ultrasonography is described in the measurement and localisation of muscle haematomata and in the third section is described the diagnosis and investigation of a patient with chronic haematomata (pseudotumour) in the pelvis resulting from untreated bleeding into the iliacus muscles.

(b) Serum enzyme changes after intramuscular
bleeding

Introduction

Creatine kinase (adenosine triphosphate: creatine phosphotransferase; CK) catalyses the reversible transfer of phosphate according to the equations:



This enzyme is found in abundance in both skeletal muscle (Oliver, 1955; Tanzer and Gilvarg, 1959) and cardiac muscle (Colombo et al., 1962). Estimation of the serum activity is widely used as a diagnostic aid in human disease (Ebashi et al., 1959; Okinaka et al., 1961; Dreyfus, 1960 (a); Dreyfus, 1960 (b)) particularly with regard to diagnosis of primary skeletal muscle disorders and in myocardial muscle damage after occlusion of the coronary arteries. Diseases of skeletal muscle which may produce elevation of serum creatine kinase are thyrotoxic myopathy, myasthenia gravis, endocrine and electrolyte disturbances (Hess, 1964) and also poisoning with hypnotic drugs (Wright et al., 1971). Serum CK levels may also be of value in the diagnosis of pre-clinical muscular dystrophy and detection of the female carriers

of the Duchenne type of muscular dystrophy (Okinaka et al., 1961; Dreyfus, 1960 (a)). High levels of CK have also been reported in patients who have suffered extensive injuries in road or industrial accidents and also following major surgical procedures (King, 1970).

A transient rise in the level of serum CK may occur after recent strenuous muscular exercise (Colombo et al., 1962; Graig and Ross, 1963; Schneider and Heise, 1963) and after severe epileptic seizures (Wilson, 1971). Similarly, elevation of lactate dehydrogenase (LDH) and aldolase has been reported after prolonged muscular exercise (Baumann et al., 1962; Remmers and Kaljot, 1963).

Relatively small amounts of creatine kinase are also found in brain, smooth muscle, kidney and liver (Colombo et al., 1962) but diseases of these tissues do not usually give rise to diagnostic levels of enzyme activity in the serum.

Changes in serum levels of creatine kinase, lactate dehydrogenase, aldolase, aspartate and alanine amino-transferases (GOT, GPT) were determined in patients with haemophilia and Christmas disease who had suffered haemorrhage into muscles either spontaneously or as a result of trauma.

Methods and materials

All enzymes were assayed by initial reaction rate procedures at 37°C. The transaminases and lactate dehydrogenase assays were carried out on the LKB 8600 reaction rate analyser using Boehringer test combination reagents. Boehringer kits were also employed for estimating aldolase and creatine kinase activities initially on a Unicam SP800, and latterly on a Unicam SP1800 recording spectrophotometer with temperature control by a circulating Heto water-bath. Enzyme activities are expressed in International Units (International Union of Biochemistry, 1961 and 1965). An addendum on the nomenclature of enzymes is found in Table 8.

Normal ranges of activities established in a hospital patient population by truncated probit analysis (Neuman, 1968) were as follows: aspartate aminotransferase (GOT) 9 - 35 IU/litre, alanine aminotransferase (GPT) 5 - 26 IU/litre, lactate dehydrogenase (LDH) 115 - 465 IU/litre, aldolase 0 - 6 IU/litre and creatine kinase (CK) 0 - 100 IU/litre at a reaction temperature of 37°C. Higher normal ranges (98 per cent confidence limits) have been obtained with the same assay procedures in a normal healthy non-hospital population giving values of 10 - 37 IU/litre for aspartate

aminotransferase, 6 - 37 IU/litre for alanine aminotransferase and 0 - 130 IU/litre for creatine kinase (McQueen et al., 1972). Electrophoretic separation of lactate dehydrogenase isoenzymes was performed as previously described (Forbes et al., 1971).

Assays of antihæmophilic factor were performed by the method as described by Margolis (1958) and modified by Breckenridge and Ratnoff (1962). Clinical grading of the severity of the defect was as described by Biggs and Macfarlane (1963).

Estimation of the serum levels of enzymes was undertaken in a series of forty-one control patients with hæmophilia or Christmas disease. They were all in-patients in hospital and had been rested in bed for at least twenty-four hours. All had some clinical bleeding problem, eg. hæmaturia, hæmoptysis, hæmarthrosis, hæmatemesis and melaena or epistaxis. None had clinical evidence of muscle bleeding or of muscle disease.

Ten patients with hæmophilia or Christmas disease with obvious or suspected muscle hæmatomata were investigated and daily estimations of the enzymes were carried out; clinical details are shown in Table 10. Serial blood samples were taken from the time of admission until the enzyme levels had returned to baseline values.

Results

Normal levels of serum enzymes in haemophilic patients without muscle haematomata:

The results are shown in Table 9. The mean level of creatine kinase is 32.7 IU/litre with a standard deviation of ± 25.2 IU/litre. When the results are shown on a histogram (fig. 16) there is a skew to the left. The upper limit of normal of CK for healthy rested volunteers in our laboratory is 100 IU/litre. The mean value in haemophilia for LDH was 271 IU/litre with a standard deviation of ± 102.4 IU/litre. The normal upper limit in healthy volunteers is 460 IU/litre. There is no significant difference in these results from the normal population.

The mean levels for GOT and GPT are shown. Five patients of the forty-one showed biochemical evidence suggestive of hepatitis and their values were eliminated from the study.

Enzyme levels in haemophilic patients with muscle haematomas

There were significant rises in creatine kinase in all ten patients. The time taken to reach the peak CK activities varied from 36 hours to 96 hours with a mean of 56 hours after onset of the haematoma. All patients showed a significant rise of CK above the baseline level within 24 hours of the onset of clinical symptoms.

Fig. 17 shows serial estimations of CK and LDH in one representative patient; the peak levels of the enzymes attained in all the patients are shown in Table 11. In three of the patients with very small muscle haematomas, the peak level of CK is relatively low and only a little above the upper limit of normal, but all started with low levels of CK activity and showed the same pattern of elevation after injury as the others. LDH was elevated above the normal range in only three patients despite serial estimations. Electrophoresis consistently showed the pattern 1 > 2 > 3 > 5 > 4. One of these patients (patient 2) had evidence of duplication of LDH₁ and this case has already been reported (Forbes et al., 1971). Serial estimations in all ten patients showed a rise in LDH activity above the baseline for that individual patient. One patient had transient elevation of GOT (patient 1) and two had transient elevation of GPT (patients 1 and 9). Aldolase was slightly elevated in patient 3.

Discussion

In this study the only enzyme in which a consistent rise was found after muscle haematoma formation is creatine kinase. The "normal" activity in serum is probably derived from muscle and in a healthy population the levels are influenced by the degree of muscular activity (Griffiths, 1966). The low levels found in the

general haemophilic population, as evidenced by the skew to the left in the histogram (fig. 16) may perhaps reflect the inactivity of these patients due to the fear of inducing bleeding and to their sedentary occupations.

Within a few hours of the occurrence of muscle bleeding, there was a rise in level of creatine kinase which reached a peak at a mean of 56 hours after onset of symptoms and persisted for a further forty-eight hours before falling to normal values by the fourth day after injury. The extent of haematoma formation in muscle was difficult to assess clinically but there appeared to be a crude positive correlation between the size of haematoma and the peak level of CK activity. LDH, GOT, GPT and aldolase showed no consistent rise after muscle bleeding.

Determination of the isoenzymes of LDH by electrophoresis was generally unhelpful. One patient (patient 1) had duplication of LDH₁ which was probably due to the large amount of plasma concentrate (cryoglobulin precipitate) infused during and immediately after surgery to repair a ruptured ureter (Forbes et al., 1971). The other five patients on whom LDH isoenzymes were estimated showed a pattern 1 > 2 > 3 > 5 > 4. This probably represents release of LDH from red cells in the haematoma; however, the pattern 5 > 4 may

possibly represent leakage of muscle LDH.

The cause of the rise in CK levels after exercise and in disease states of muscle is not known. It has been suggested that it is due to leakage of the enzyme through the sarcolemma and it has been further suggested (Aebi et al., 1962) that CK is more likely to pass to the extracellular compartment than the other muscle enzymes, because of its high concentration and low molecular weight (Schmidt and Schmidt, 1967). In muscle haematomata the cause of the rise in CK is not known. It is uncertain whether actual cell death must occur to liberate CK but it has been shown in vitro that changes in cellular permeability may permit the escape of other intracellular enzymes (Schmidt et al., 1963).

The majority of patients in this series had muscle haematomata which were clinically obvious and presented no diagnostic problems. Estimation of CK, however, shows such a consistent elevation following muscle bleeding that it may be of value in the haemophiliac who presents with abdominal pain of obscure aetiology. Case 10 illustrates the potential usefulness of CK estimation as a diagnostic test. This patient presented with severe loin pain which was initially diagnosed as being due to pyelonephritis. However, the urine was sterile and both an isotope renogram and an intra-

venous pyelogram were normal. After infusion of plasma, pain stopped and the patient was sent home. Similar symptoms recurred two weeks later and a pattern of enzyme change similar to that seen in association with muscle haematoma formation was found (fig. 18). The diagnosis would seem to have been bleeding into the sacrospinalis muscle.

It is suggested that estimation of creatine kinase may be of value in the diagnosis of occult muscle haemorrhage in patients with bleeding disorders and it is possible that it may be of value in the differentiation of acute appendicitis from haemorrhage into the ileo-psoas muscle, a recurrent clinical problem in haemophilia.

(c) Ultrasonic investigation of haematomata

Introduction

Ultrasound techniques have been used in diagnosis since 1945 when Dussik tried to measure the transmission of ultrasonic energy through the intact brain. The sound used has a frequency in the order of 1 - 10 million cycles per second. It is produced in pulses of a few microseconds from a transducer and is then passed into the tissue under investigation as a narrow beam. Different tissues transmit and reflect ultrasound differently according to

their physical properties, so that when the interfaces between the constituent elements of the medium are concerned, a small proportion of the sound is reflected. On returning to the transducer, the reflected energy produces an electronic pulse which can be displayed on a cathode ray oscilloscope. Simultaneous display of many such echoes as finite spots enables a correctly relationed two-dimensional image to be displayed and this is recorded on Polaroid film. The image is produced by reflection of sound from interfaces within the tissues. By moving the probe with an angular rocking movement around the area under investigation a tissue may be examined from a variety of directions to produce the compound "B" scan.

Ultrasound has been used as a diagnostic aid in a variety of clinical situations eg. differentiation of gynaecological tumours (Donald et al., 1958), diagnosis of intracerebral lesions (Jefferson, 1959), obstetrics (Willocks et al., 1967; Willocks and Dunsmore, 1971) and in diseases of the kidney and bladder (Mountford et al., 1971; Barnett and Morley, 1971). The present study was undertaken to evaluate the use of ultrasonography in the diagnosis and

localisation of haematomata in patients with bleeding disorders.

Materials

Seven patients were examined with clinical haematomata of muscle. All had haemophilia or Christmas disease of a severe grade and one patient had a circulating anticoagulant. The clinical details are shown in Table 12.

The machine used in this study was a "Diasonograph" scanner with a swept gain facility such that echoes arising from deep structures were amplified to compensate for the absorption of acoustic energy in tissue.

The skin was liberally coated with arachis oil to provide acoustic coupling and compound "B" scans were performed in the longitudinal and transverse planes. The scans were performed in parallel to imaginary lines drawn through fixed anatomical landmarks, usually bony prominences.

The machine sensitivity was adjustable and machine settings were recorded on the back of each scan.

Results

A selection of Polaroid photographs from the patients is shown in figs. 19 (a) (b), 20 (a) (b). All ultrasonograms showed that the haematomata were poorly transonic i. e. behaved as cystic

lesions and were of varying consistency. The extent of the lesion was clearly defined both longitudinally and transversely and as the reduction factor is 1 : 5 on the Polaroid picture this allows actual measurements of the haematoma size. All haematomata contained reflecting speckles.

Discussion

Pulsed ultrasound provides a reasonably simple, safe and repeatable diagnostic technique but it has not been used before in the investigation of haematomata. In our hands it has allowed accurate localisation and measurement of haematoma size and provided an objective method of following resolution with therapy.

It seems probable that the interface of muscle and haematoma consists of compressed muscle fibres and it may be that the "speckles" within the haematoma consist of degenerating muscle fibres, nerves and blood vessels. It is apparent that in some cases (fig. 21) the haematoma remains localised and does not expand to fill the muscle belly. This may be a result of clotting of the extravasated blood by liberated tissue thromboplastin.

With therapy all these haematomata rapidly resolved and left no apparent residual damage. Repeat ultrasonography showed muscle echoes which were diminished in volume compared to the normal side.

The fate of the blood clot is not known. Presumably the red blood cells are digested by local tissue and white cell enzymes and the haemoglobin denatured to bilirubin which is then excreted by the liver. The fibrin clot is presumably also digested in this manner as in no patient was there a significant elevation of fibrinogen degradation products (FDPs, FR-antigen) in the serum (Forbes and Prentice, 1972).

Ultrasonography, therefore, offers a rapid effective method in the delineation of muscle haematomata and in assessment of their resolution.

(d) Bilateral pseudotumours of the pelvis in a patient with Christmas disease: with notes on localisation by radioactive scanning and ultrasonography.

Introduction

Pseudotumours in patients with coagulation defects are rare, the incidence in several large series of haemophilic patients varies from 0.5 to 2 per cent (Ahlberg, 1965; Wessler and Avioli, 1968; Gunning, 1966; Bayer et al., 1969). The most common sites are the thigh and the pelvis but a small number of cases have been reported in the calves, feet, arms and hands. Bi-

lateral pseudotumours have been recorded in only two patients; these involved the cuboid bones of the feet (Chen, 1965) and the pelvis (Fraenkel et al., 1959).

Pseudotumours are found in both haemophilia and Christmas disease (Ahlberg, 1965; Revol, 1965; Silber and Christensen, 1959; Bayer et al., 1969) despite the suggestion that Christmas disease patients tend to have a less serious defect (Ratnoff, 1960; Ramgren, 1962). It is of great interest that a pseudotumour has also been reported in a patient with a mild bleeding history and an antihæmophilic factor assay of 23 per cent (Pappas et al., 1964).

In several of the case reports, including the original, there has been doubt about the diagnosis, the suspicion being that the destructive lesion might be osteogenic sarcoma (Starker, 1918; Becker, 1942; Nelson and Mitchell, 1962; Pappas et al., 1964). In many of the cases where biopsy was undertaken serious complications ensued including death from infection introduced at the time of the diagnostic aspiration or uncontrollable hæmorrhage from the needle track (Starker, 1918; Abell and Bailey, 1960; Nelson and Mitchell, 1962; Silber and Christensen, 1959). Osteogenic

sarcoma has been described in a haemophilic patient (Fraenkel et al., 1959) and should always enter into the differential diagnosis despite its rare occurrence.

We describe here the case of a boy with severe Christmas disease with bilateral pseudotumours in the pelvis. We have used radiological techniques and ultrasonography to localise the tumours. In addition scintiscanning was carried out using ^{87}m strontium, a bone seeking radionuclide and ^{99}m technetium which localises in tissues with large or abnormal vasculature. Scintiscan pictures of known osteogenic sarcoma were compared with the appearances of the pseudotumours.

Methods

For scintiscanning ^{87}m strontium (2 mCi) was given intravenously and 55 minutes later the lower abdomen, pelvis and upper femora were scanned in that order using a dual headed scintiscanner. A colour dot and photo scan was obtained. On a separate occasion a similar procedure was carried out 20 minutes after ^{99}m technetium (10 mCi) had been given intravenously.

Ultrasonography was performed using a Nuclear Enterprises Diasonograph.

Case Report The patient is now aged twenty-five years and has a severe degree of Christmas disease with a zero per cent assay of Christmas factor. In his youth he experienced multiple bleeding episodes, involving especially the joints, renal tract and after dental extraction. When aged nine years he developed a painful tense swelling in the left groin associated with numbness down the front of the left thigh. He was seen in hospital at this time and the presence of an iliacus haematoma with compression of the femoral nerve was confirmed. X-ray of the pelvis showed a homogeneous soft tissue mass measuring 9 cms in diameter overlying the left iliac crest, encroaching the upper part of the sacro-iliac joint as well as the distal third of the left psoas muscle with no bone erosion. Treatment with plasma was given for a week with some slight resolution of the mass but the patient then defaulted from further attendance.

In 1963 he developed a similar pain and swelling in the right groin with a femoral nerve palsy which was treated in his home without plasma infusions. In 1964 he had a recurrence of pain in the same site and thereafter was reasonably well except for recurrent episodes of haematuria and acute haemarthroses of

knees, ankles and shoulders. The patient was readmitted to hospital in 1971 having fallen and sustained a fracture of the right tibia and the left patella.

Examination of the abdomen showed two large swellings arising out of each side of the pelvis. Both were hard, non-tender and non-fluctuant. The swelling of the left was largest, extending to just above the umbilicus. There were bilateral femoral nerve lesions with absent knee jerks and gross wasting of both quadriceps.

Plain X-ray films of the pelvis showed erosion of the blade of the ilium on the left side. Continuous with this area of bone destruction there was a soft-tissue mass within which were areas of calcification. Osteosclerotic areas were also seen in the ilium (fig. 22). On intravenous pyelography it was seen that the bladder and left ureter were displaced to the right. The appearance of the lower end of the left ureter suggested further that it was held in fixed dilatation as a result of its involvement by the pseudotumour (fig. 23(a)). A barium meal and follow through confirmed the appearances previously noted, namely that both small and large bowel were displaced by the large left-sided

pseudotumour and also by a smaller but presumably similar lesion on the right side (fig. 23(b)).

At this stage ultrasonography was performed with a view to confirming that there were in fact two separate masses. It was also considered that ultrasonic scanning would constitute a simple and safe method of assessing the effects of therapy on the size of the lesions. As expected, two discrete lesions were shown (fig. 24).

Examination of the ultrasonographs showed that these lesions were poorly trans-sonic i. e. were fairly solid structures, and were of varying consistency. This together with their rather indistinct margins, makes them indistinguishable from malignant tumours such as an osteogenic sarcoma.

The scan pictures after ^{87}m strontium and ^{99}m technetium were similar and only the ^{87}m strontium photo-scan of pelvis is shown (fig. 25). This shows a large round volume of abnormal uptake in the right pelvis and an elongated volume of abnormal uptake extending laterally and superiorly in the left pelvis. Both regions correspond to the sides of the pseudotumours. The general appearances are similar to those we have found in known femoral osteogenic sarcoma.

Discussion

At least forty-nine cases of pseudotumour in haemophilia have been recorded since the original case report of Starker (1918) and of these twenty have involved the ilium (Forfota, 1931; Peterson, 1947; Parks, 1956; Horowitz et al., 1959; Fraenkel et al., 1959; Silber and Christenson, 1959; Birk, 1960; Schwarz, 1960; Nelson and Mitchell, 1962; Hall et al., 1962; Kerr, 1963; Caen et al., 1964; Ahlberg, 1965; Goulston and McGovern, 1965; Eibl et al., 1965; Revol, 1965; Wessler and Avioli, 1968).

As suggested by Valderama and Matthews (1965) haemophilic haematomas may arise in muscle where they are confirmed by the tendinous attachments (simple cyst), or in a muscle with a large periosteal attachment and produce cortical thinning by interference with bone nutrition; alternatively they may arise from a haematoma which strips the periosteum from the cortex until limited by an aponeurotic or tendinous attachment. It is difficult, in retrospect, to determine the site of origin of this patient's pseudotumours but the initial episodes of pain and femoral nerve compression suggest strongly that they started as haematomata in the iliacus muscle (Goodfellow et al., 1967). The lesion on the left has

probably been present for sixteen years and the lesion on the right for eight years. As judged both clinically and radiologically the left pseudotumour has grown over the years but it is not possible to say whether this has occurred gradually or whether it is still enlarging. Serum enzymes levels were normal. It seems probably from the history that the two tumours have arisen from separate sites and both the ultrasonic and photo scans suggest that they are discrete entities.

Although renal function, as judged by the blood urea of 25 mg/100 ml and the creatinine clearance of 127 ml/min is excellent, there is evidence both from the I.V.P. and the isotope renogram of obstruction to the ureters. Similarly there is as yet no interference with bowel function but the barium meal and follow through shows distortion of the normal anatomy of the large and small bowel in the pelvis. There is one case report of pseudomembraneous colitis in a haemophiliac associated with pseudotumour of the pelvis producing obstruction to the rectum (Goulston and McGovern, 1965) and one of rupture into the sigmoid colon (Hall, 1961). There has been no case, to date, of renal failure resulting from compression of the ureters.

Treatment of the pseudotumour can be either medical or surgical. Medical management has been advocated by several authors (McMahon and Blackburn, 1959; van Creveld and Kingma, 1961; Caen et al., 1964; Favre-Gilly et al., 1965). Correction of the coagulation defect with long term prophylaxis resulted in resolution of the haematoma in one case (McMahon and Blackburn, 1959) and prevented enlargement in another (Kerr, 1963). Success of this treatment probably depends on the size of the haematoma as well as how long it has been present (Fraenkel, 1957). Long term plasma or plasma concentrate therapy carries its own hazards of transmission serum hepatitis or production of inhibitors to antihæmophilic or Christmas factor. Irradiation of these tumours has fallen into disrepute (Horowitz et al., 1959) despite the initial encouraging reports of Muller (1942) and Chen (1965).

When the lesion is peripheral then surgery has a better chance of success (Britten and Salzman, 1966) particularly when amputation of the limb is carried out. Surgery has been successfully performed on pseudotumours of the pelvic region (Hall et al., 1962; Bailey et al., 1965; Wessler and Avioli, 1968), but because of their size, position and proximity to vital structures

the results have been poor. Steel et al. (1969) however advocate radical surgery for iliac pseudotumours but we feel the only hope of treating this patient is with long term infusions of Christmas factor concentrate. This has now been given for four months but no change in size of the tumour has been noted either clinically or on ultrasonography.

From studies of the operative case notes of the patients undergoing excision of these tumours they appear to be extremely vascular and present major technical problems of haemostasis. Macroscopic and histological examination confirms the presence of large vessels in the outer fibrous zone of the capsule (Fraenkel et al., 1959; de Valderrama et al., 1965) and this would explain the localisation of both ^{87}m strontium and $^{99\text{m}}$ technetium in and, especially around, the tumour in the pelvis.

Both ultrasonography and radioisotope scanning have failed to differentiate the pseudotumour from an osteogenic sarcoma but they offer methods of precise localisation and measurement of the size of the tumours and should provide an objective assessment of the efficacy of plasma therapy.

CHAPTER 5DENTAL EXTRACTION IN HAEMOPHILIA(a) Introduction

Dental extraction is a necessary evil in the life of every haemophiliac patient and early case reports in the literature are spiced with dramatic tales of exsanguination and death after extraction of a single tooth (Davies, 1826; Craig, 1826); for example, the medical student whose case was recorded by Coates (1828) had a single tooth extracted and lost half a gallon of blood in twenty-four hours; the bleeding lasted ten days and the entire quantity of blood lost was estimated at "not less than three gallons". Because of these problems dental extraction was specifically contraindicated (Legg, 1872). Exsanguinating haemorrhage from such a small site obviously tried the patience and ingenuity of generations of physicians who used many preparations of chemicals (mercury, silver nitrate, alum, gallic acid, turpentine, strychnine, tannin, perchloride of iron) and many surgical procedures (phlebotomy, the red hot iron, suturing of the socket, pressure from a cork or a bullet, arterial compression) with little success. Unfortunately all these methods were doomed to failure and indeed some

exaggerated the problem; death is recorded after therapeutic phlebotomy and massive haemorrhage always occurred when the sloughs separated after use of "the red-hot iron".

The first major advance came with the introduction of blood transfusion (Weil, 1906). Unfortunately, blood used for transfusion purposes contained only small amounts of anti-haemophilic factor and it was not unusual for a patient to remain in hospital for 6 - 8 weeks and require twenty pints of blood after extraction of a single tooth. With the advent of plasma therapy (Feissley, 1924) dental extraction became less dramatic but still extractions of many teeth simultaneously lead to excess bleeding. Many types of splints were devised to protect the healing socket and prevent damage to the friable blood clot (Wishart et al., 1957). These probably provided some advantage but were uncomfortable to wear and, if ill-fitting, produced ulceration and haemorrhage at the alveolar margin.

With the advent of potent concentrates of both human and animal antihemophilic factor tooth extraction became a routine, trouble-free event, even in patients with circulating anti-coagulants (Biggs and Macfarlane, 1966). Awareness has since

increased of the problems of serum hepatitis and circulating anticoagulants to AHF induced by the infusion of plasma products and a search has been made for agents which might reduce transfusion requirements.

It is thought that, in haemophilia, bleeding from the tooth socket is due, in part, to an imbalance between the deposition of fibrin clot and digestion of this clot by the enzymes of the fibrinolytic system. Attempts have therefore been made to promote haemostasis by inhibition of fibrinolysis using the antifibrinolytic drug aminocaproic acid (EACA, Epsikapron (Reid et al., 1964; Cooksey et al., 1966; Tavenner, 1968; Walsh, et al., 1971).

These trials of EACA have, however, been largely uncontrolled and the criteria of efficacy of this drug have also varied from trial to trial, eg. Cooksey et al. (1966) and Tavenner (1968) used transfusion requirements and days spent in hospital; others (Alagille et al., 1965; Marini et al., 1966; Giordano et al., 1967; Paddon, 1967) compared visible blood loss and some (Reid et al., 1964) used the fall in the haemoglobin level. Walsh et al. (1971) in their well-controlled, double blind trial used the amount of plasma concentrates given as the index of benefit. We

describe here a double blind trial of tranexamic acid in dental extraction in patients with haemophilia and Christmas disease in which measurement of the blood loss was carried out using ^{51}Cr labelled red blood cells. Observations were made at the same time of possible toxic effects of tranexamic acid.

(b) Methods and material

Design of the trial

Twenty-eight patients aged from thirteen years to sixty-five years were studied during thirty-two separate episodes of dental extraction. Twenty patients had classical haemophilia and eight had Christmas disease.

Informed consent was obtained from all the patients or from the parents before they were included in the trial. A history of haematuria in the previous four weeks, or the presence of red cells in a fresh sample of urine, were absolute contraindications to inclusion in the trial.

Using a double blind technique and random allocation to the trial the patients received either tranexamic acid (1 gm three times per day) or placebo tablets. Therapy was started two hours before extraction and continued for a five day period.

Blood loss was measured using ^{51}Cr labelled red blood cells as described by Watson and Dickson (1964). This entailed separate collections of oral secretions and faeces over 24 hour periods for five days. On day 4 the patient received a purgative.

Each patient received the factor VIII or IX equivalent of 1000 ml of human plasma intravenously one hour before extraction and also tetracycline (250 mg four times per day). Infection at the site of extraction is thought to be a contributing factor to contribution of bleeding. All extractions were carried out under local anaesthetic. In the event of excess bleeding from the sockets in the five day period, a clinical decision was made to infuse further blood products. Sufficient plasma or plasma concentrate was given only to stop the bleeding. The clinician was not aware which tablet the patient was receiving, nor the results of the laboratory assays.

Factors VIII and IX were assayed using the method of Margolis (1958) as modified by Breckenridge and Ratnoff (1962). Tests of fibrinolysis were performed as previously described by McNicol and Douglas (1964) and assessment of parameters of liver and renal function, were performed by standard Autoanalyser techniques. Haematological indices were measured on the Coulter-S

counter and erythrocyte sedimentation rate by the method of Westergren (1920). Inhibitors of factor VIII and IX were sought using the method of Biggs and Bidwell (1959). Standard 12 lead electrocardiographs were made in all patients before and at the end of the trial. All patients in this series had the extractions performed under local anaesthesia, including where appropriate, inferior dental block. No side effects resulting from this procedure were observed in any patient. Details of the patients in both groups are shown in Table 13.

Results

The data concerning blood loss is shown in Table 14.

Teeth extracted:

As the amount of bleeding is partly a reflection of the extent of the wound area, the number of roots removed is considered rather than the number of teeth removed. The number of roots extracted in the tranexamic acid group (mean 5.5) was not significantly different from the placebo group (mean 6.9).

Blood loss:

The mean volume of blood lost per patient in the placebo group was 85 ml and in the tranexamic acid treated group 61 ml.

There was a wide range in the total loss per patient. The blood loss per root extracted was 15.3 ml/root in the placebo group and 8.9 ml in the tranexamic acid group. This corresponded to a mean fall of 1.4 gm per 100 ml in the haemoglobin level and 5 per cent fall in haematocrit in the placebo group and a fall of 0.3 gm per 100 ml in the haemoglobin and a 0.9 per cent fall in haematocrit in the tranexamic acid group. Using a rank sum test (Mann-Whitney U test) there is a statistically significant difference between the blood loss in the placebo group and the AMCA group ($0.01 < p < 0.025$).

Replacement therapy:

The results are shown in fig. 26. In only two patients was it clinically necessary to transfuse plasma or plasma concentrate in the tranexamic acid group after the initial dose. One of these patients had extraction of twenty-two roots, the largest number in this series. In the placebo group eleven patients required multiple infusions during the five day trial period and five patients required no replacement therapy; all these had a mild or moderate degree of defect. The patient requiring the greatest amount of replacement therapy was in the moderate degree of

haemophilia and had six roots extracted. He was in the placebo group.

Plasma fibrinogen levels and erythrocyte sedimentation rate:

The results are shown in fig. 27. Significant elevation of fibrinogen is found in the placebo group on day 5 after extraction. This finding is mirrored in the erythrocyte sedimentation rate which is also significantly increased ($p = 0.05$).

Urokinase sensitivity and euglobulin lysis time:

The results are shown in fig. 28. As would be predicted, significant depression of lysis is shown in the urokinase sensitivity test after treatment with tranexamic acid ($p = 0.05$). Both treated and untreated groups show a fall in the euglobulin lysis times indicating increased levels of circulating activator. The inter group results are, however, not significantly different ($p = 0.1$); the tranexamic acid is discarded in the supernatant during preparation of the euglobulin precipitate.

Renal and liver function tests:

Estimations included serum urea, bilirubin, alkaline phosphatase, S.G.O.T., S.G.P.T., total proteins, albumin and globulin. No significant differences were found between

the tranexamic acid treated group compared with placebo.

Both groups showed a significant rise in the blood urea after extraction ($p = 0.05$).

Electrocardiograph:

No changes are found after therapy in either group.

Side effects:

No side effects were reported in either group.

(c) Discussion

It has been shown by many authors that surgery can be safely undertaken in haemophilia and Christmas disease if adequate replacement therapy with plasma products is provided. The criteria for success of a new therapeutic agent in dental extraction must include a reduction in blood loss from the extraction site and also a reduction in the total requirement for plasma replacement therapy. The use of tranexamic acid as an adjunct to conventional therapy in this series of dental extraction fulfilled both criteria; post-operative bleeding and also the total volume of plasma product required, were both reduced.

Tranexamic acid is the active trans-stereoisomer of aminomethyl cyclohexane carboxylic acid and it has been shown

by Melander et al. (1964) and Okamoto et al. (1964) to have powerful anti-fibrinolytic properties. The molecule has a configuration similar to aminocaproic acid and like aminocaproic acid is a competitive inhibitor of plasminogen activation at concentrations in plasma greater than 10^{-4} M and a non-competitive inhibitor of plasmin at concentrations greater than 10^{-2} M. These concentrations may be readily achieved in vivo (Dubber et al., 1965).

Tranexamic acid had the advantage over aminocaproic acid of being about ten times more potent (Dubber et al., 1965; Melander et al., 1965; Deutsch and Fischer, 1968) and having minimal side effects. All inhibitors of fibrinolysis when used in patients with haemorrhagic disease have the potential danger of precipitating obstruction of the renal tract with unlysable clots (McNicol et al., 1961; Gobbi, 1967; Van Itterbeek et al., 1968) however no patients in this study, who were all screened prior to therapy for the presence of haematuria, showed evidence of this complication.

Although the cause of post-extraction bleeding from the tooth socket in the haemophiliac is not clear, it presumably

represents an imbalance between defective fibrin formation due to the abnormality in the intrinsic thromboplastin system and the normal removal of deposited fibrin by fibrinolytic enzymes produced by plasminogen activators locally and in the saliva (Albrechtson et al., 1958). It was noted that all patients in this series showed a fall in the euglobulin lysis times, indicating increased levels of circulating plasminogen activator. This has been recorded by several authors in people under stress (Macfarlane, 1937; Biggs et al., 1947) and may be important in the light of the report by Lucas et al. (1962) that extraction of teeth may be safely carried out in haemophiliacs under hypnosis without plasma therapy. However, in the immediate post-extraction period the most important aspect of haemostasis is probably defective fibrin formation. In a recent study (Walsh et al., 1971), haemophilic patients who had their level of clotting factor raised to 50 per cent of normal by a single haemostatic infusion before extraction rarely bled before the third post-operative day. This suggests that despite the transient nature of the rise in the plasma level of these factors the haemostatic effect is prolonged. Bleeding after three days (up to six days) was commonly seen in our group of control patients and presumably represented digestion of

the haemostatic fibrin plug by fibrinolytic enzymes. Inhibition of these lytic enzymes by tranexamic acid, as was manifested in the prolongation of the urokinase sensitivity tests, allowed fibrin to remain in situ and presumably expedited healing of the sockets. This greatly reduced the necessity to infuse plasma products into our patients.

We consider that measurement of blood loss using ^{51}Cr labelled red blood cells offers a more accurate assessment of the effectiveness of therapy than fall in haemoglobin or days in hospital. However, in all patients, when bleeding was noted clinically, plasma or plasma products were immediately given and the effectiveness of tranexamic acid must be judged against a combination of total blood lost and the amount of plasma products infused.

There was no evidence of any toxic action of tranexamic acid in this series of patients. There was however a significant rise in the mean blood urea levels in both groups after tooth extraction. This probably results from digestion of blood in the gut.

The complications of multiple transfusions are well known (de Cruchy, 1962). In haemophilia these may include production of inhibitors of antihæmophilic factor and serum hepatitis. It is a reasonable assumption that reduction of plasma requirements by the use of tranexamic acid will reduce the incidence of inhibitors and serum hepatitis as well as reducing blood loss.

CHAPTER 6GASTROINTESTINAL BLEEDING IN HAEMOPHILIA(a) Introduction

In his review of 256 cases of bleeding disorders Grandidier (1855) found gastrointestinal haemorrhage to be the second commonest cause of spontaneous bleeding and this observation has been confirmed by Wilkinson et al. (1961) and Stuart et al. (1966). Many isolated case reports have appeared of the surgical management of bleeding associated with proven peptic ulceration in haemophilia but no large series of cases of gastrointestinal haemorrhage was published until 1965 (Carron et al., 1965). At that time about one third of deaths in the haemophilic population resulted from uncontrolled intestinal haemorrhage or its sequelae (Nour-Eldin, 1961; Wilkinson et al., 1961).

In many cases no local source of bleeding was found (Britton, 1963) but in some the cause of bleeding was peptic ulceration (Desmond, 1955; Walker, 1955; van Creveld, 1957; Macfarlane et al., 1957; Handley et al., 1961; Ingram, 1962; Kerr, 1964(a)). In all of these case reports operative intervention was necessary because of massive haemorrhage and all

required some variety of gastrectomy.

The incidence, clinical features and sequelae of gastrointestinal haemorrhage in haemophilia have been well documented by Carron et al. (1965). They made a retrospective study of the case records of eighty registered adult haemophilic patients in Newcastle and found fourteen out of eighty had suffered gastrointestinal haemorrhage, twelve of these had a duodenal ulcer. Six of the patients with duodenal ulcer required gastrectomy and one pyloroplasty and vagotomy. It was the conclusion of these authors that if a patient with haemophilia is found to have peptic ulceration he should have elective surgery after the first gastrointestinal bleed.

In the last few years there have been dramatic changes in the availability of potent concentrates of plasma to treat haemophilia and Christmas disease so it seemed worthwhile to reassess the prognosis of gastrointestinal haemorrhage in these bleeding disorders.

(b) Patients and methods

All patients with haemophilia or Christmas disease in the region who have had haematemesis or melaena in the past ten years

have been included in the survey. Clinical grading of severity of the bleeding defect was that described by Biggs and Macfarlane (1963) and assay of the antihæmophilic factor or Christmas factor was performed by the method of Margolis (1958) as modified by Breckenridge and Ratnoff (1962). All patients underwent barium meal examination in an attempt to determine the site of bleeding. Duodenal or gastric ulcer were diagnosed only when an ulcer crater was visualised on the barium meal and confirmed in the patients who underwent surgery.

Results

Thirty-two patients with 107 separate episodes of gastrointestinal bleeding were found. This represents an incidence of 25 per cent in the adult hæmophilic population. Twenty-seven of the patients had classical hæmophilia and five had Christmas disease. Fifteen had a severe defect, twelve were moderately affected and five were mildly affected. At the time this survey was undertaken the mean age of the patients was 40.1 years the youngest being twenty-two years and the oldest sixty-six years.

The average age of onset of the first gastrointestinal hæmorrhage was 31.6 years but there was a wide range, the

youngest patient bleeding at age 6 years and the oldest at 63 years. When the age of onset of the first symptoms of gastrointestinal disease is considered (dyspepsia or bleeding) the mean age was 30.6 years. This difference was not significant. In patients with dyspeptic symptoms alone the average age of onset was 30.8 years.

The patients were then classified into two main groups according to whether they had symptoms of dyspepsia suggesting a clinical diagnosis of peptic ulceration. The first group consisted of seventeen patients who had a history of dyspepsia and the second group of fifteen patients had no history of dyspepsia. This is obviously an artificial division but from the clinical point of view it serves to separate the patients. The relevant clinical details of the two groups are shown in Tables 15 and 16.

Age

There is no significant difference between the ages of these two groups, the mean age in the eupeptic group being 38.5 years (range 22 - 66) and the mean age in the dyspeptic group 40.9 years (range 17 - 60).

Severity of haemostatic defect

There is a significant difference between the two groups when the severity of the haemostatic defect is considered. As seen in Table 3 the eupeptic group contains eleven patients with a severe defect, three with a moderate defect and one mild defect. In the dyspeptic group there are four with a severe defect and nine with a moderate defect and four mild cases. The differences in numbers of the severe and moderate patients are highly significant ($\chi^2 = 4.5$, $p < 0.05 > 0.02$).

Blood group

In the thirty-two patients there are eighteen with blood group O (56.25 per cent), nine with blood group A (28.2 per cent), three in group B (9.4 per cent) and two in group AB (6.15 per cent). When the seventeen patients with radiologically proven peptic ulcer are considered there are eight in group O (47 per cent), six in group A (35.3 per cent), two in group B (11.8 per cent) and one in group AB (5.9 per cent). These results are not significantly different from the incidence of blood groups in the West of Scotland.

Age at which first bleeding occurred

There was no significant difference between the groups in the age at which bleeding occurred. This was 31.4 years (range 6 - 50) in the eupeptic group and 31.6 years in the dyspeptic group.

In the dyspeptic group clinical symptoms suggestive of peptic ulcer preceded bleeding in eleven patients and in three symptoms started at the time of the first bleeding episode. In three other patients symptoms did not appear till after the first gastrointestinal haemorrhage. In the eleven patients with dyspepsia preceding bleeding there was an average period of 4.6 years (range 1 - 13 years) with ulcer type symptoms before haemorrhage.

Number and type of bleeding episodes

In the thirty-two patients studied, a total of 107 episodes of gastrointestinal bleeding were recorded. Eighty episodes of bleeding started with haematemesis and twenty-seven with melaena. In the seventeen patients in the dyspeptic group there were fifty-eight episodes of bleeding (mean 3.4, range 1 - 15) and in the eupeptic group of fifteen patients a total of forty-nine

bleeding episodes (mean 3.3, range 1 - 14). In patients with proven peptic ulcer the mean (4.5) number of bleeds is higher than the mean for the rest of the patients (2.1). This result is significant ($t = 2.61$, $p < 0.01$).

Association of bleeding with salicylates or alcohol

Fourteen of the 107 episodes of bleeding were associated with prior ingestion of salicylate or alcohol. In both groups there were four episodes associated with salicylate ingestion and three with alcohol ingestion. Patient 3 was admitted with haematemesis on two occasions after drinking heavily and on three occasions after taking salicylates.

Time between onset of bleeding and diagnosis

In the dyspeptic group four patients were shown on barium meal examination to have peptic ulceration before the first bleed but nine had no radiological proof of ulcer until some years after the first bleed (mean length of time = 5.4 years, range 6 months - 12 years) despite having ulcer symptoms. Two had positive barium meals at the time of bleeding and in two others no bleeding source has been found. In only nine of the thirty-two patients

was the probable source of bleeding shown radiologically at the time of the first episode of haematemesis or melaena.

Diagnosis

In the dyspeptic group, fifteen of seventeen patients had peptic ulceration; twelve had duodenal ulceration, two gastric ulceration and one both duodenal and gastric ulcers.

In the eupeptic group, eleven patients had normal barium meals, two had radiological evidence of active duodenal ulceration, one had a haematoma of the duodenal wall and one had intestinal obstruction with volvulus of the ileum as a result of an ileal haematoma. Patient 6 had chronic myeloid leukaemia and was on busulphan treatment at the time of bleeding but was not thrombocytopenic. One patient (number 11) exsanguinated from alimentary bleeding despite massive infusions of blood (150 pints), plasma, cryoprecipitate and porcine antihaemophilic factor. No radiological lesion was demonstrated. He was shown to have a potent antihaemophilic factor inhibitor and at post mortem was found to have multiple erosions throughout the length of the gastrointestinal tract.

Surgical management

Seven patients have undergone surgery. Only one patient required an emergency procedure. The details are shown in Table 4. Four patients (7, 12, 20 and 29) had pyloroplasty and vagotomy for duodenal ulceration and two (patients 19 and 31) underwent partial gastrectomy for gastric ulcers. Only one patient has done badly after surgery (patient 7). He has had eight haematemeses post-operatively all of which have been treated conservatively. Insulin test meal has shown that vagotomy is incomplete the pH of the gastric secretion falling to pH1. Two other patients (20, 31) have symptoms of mild dumping which have responded to conservative management. Patient 30 developed a potent antihæmophilic factor inhibitor which was demonstrated on the seventh post-operative day and patient 12 developed serum hepatitis as a result of cryoprecipitate therapy given during the operative period.

Discussion

Gastrointestinal bleeding is a common feature of hæmophilia and Christmas disease but it may be extremely difficult in the individual patient to demonstrate the basic pathological lesion.

In this survey we have shown that 25 per cent of our adult haemophilic patients have had gastrointestinal bleeding. Of these 53 per cent have peptic ulceration, gastric or duodenal. The incidence of proven duodenal ulcer is 13.2 per cent of the adult haemophilic population, an incidence which is significantly higher than that found by Doll and Avery-Jones (1951) and Illingworth (1953) in the general population. It is well established that this group of patients are subjected to abnormal stress (Early, 1959; Agle, 1964) and this may precipitate a bleeding episode (Legg, 1872; Browne, Mally and Kane, 1960; Mattsson and Gross, 1960). It is also possible that the delayed healing in haemophilia, such as is seen with a tooth socket, may also play a part in the continuation of bleeding.

There is a significant difference in incidence of severely affected patients in the eupeptic and dyspeptic groups. In the eupeptic group, where there is a preponderance of severely affected patients, it is possible that bleeding occurs readily when the mucosal injury is minimal, whereas in the dyspeptic group, where the majority of patients are only moderately affected, the mucosal lesion must be more advanced before bleeding occurs.

The absence of symptoms in the presence of active duodenal ulcers is well known. Dunn and Etter (1962) found evidence of duodenal ulceration radiologically in 31 per cent of 206 patients who had no ulcer symptoms. Duodenal ulceration was shown radiologically in two of our patients (7, 16) who have had no symptoms.

The average age at which the first gastrointestinal bleed occurred was 31.6 years in the whole group and 29 years in the patients who were eventually shown to have duodenal ulcer. This is slightly older than found by Carron, Boon and Walker (1965) in haemophilia and also by Doll and Avery-Jones (1951) in the general population. In the patients with dyspeptic symptoms the finding of an average of 4.6 years history before the first haemorrhage is similar to that found in patients with peptic ulcer in the general population by Coghill and Willcox (1960).

There is a preponderance of blood group O in this series of patients (56.5 per cent) but this is not significantly different from the frequencies of ABO groups in the West of Scotland (Wallace, 1954). There is no relationship between ABO blood groups and haemophilia (Chaudhuri, 1960; Wilkinson et al., 1961)

but there is a positive correlation between peptic ulcer (especially duodenal ulcer) and blood group O (Aird et al., 1954) and also between liability to haemorrhage and blood group O (Langman and Doll, 1965). A predominance of blood group O in haemophilic patients with gastrointestinal bleeding has been reported previously by Carron et al. (1965).

Ingestion of aspirin and aspirin containing components produces a defect in aggregation of platelets (Zucker and Peterson, 1968) and in patients with coagulation defects may produce severe haemorrhage (Kaneshiro et al., 1970). All our patients with coagulation defects have been warned about the effects of salicylates and this probably accounts for the low incidence of salicylate induced bleeding in this series compared with patients with non-haemophilic haematemesis (Alvarez and Summerskill, 1958).

The recurrence rate of gastrointestinal haemorrhage is high; twenty-three of the patients have had more than one episode and one has had fifteen separate episodes. All of these recurrences have been treated conservatively with plasma concentrate and blood if necessary. No patient has required emergency surgery for intestinal bleeding per se; patient 30 required emergency surgery

for intestinal obstruction due to volvulus of the ileum. Only one patient died of haemorrhage and he had been shown to have a potent antihaemophilic factor inhibitor. This low mortality is in keeping with the figures of Fraenkel and Truelove (1955) for non-haemophilic haematemesis in which the prognosis was shown to be dependent on the age of the patient. This low mortality shows an improvement from the 33 per cent mortality in haemophilic gastrointestinal bleeding quoted by Nour-Eldin (1961) and it would seem likely that this is due entirely to the availability of potent concentrates of the appropriate coagulation factor.

The decision to advocate surgery is extremely difficult and on balance we still tend to take a conservative approach. The criteria for surgery in non-haemophilic patients with peptic ulcer (Hunt, 1952) do not apply to the haemophiliac. In the small series treated surgically three patients have had an excellent result, two have mild dumping syndrome and two have had further gastrointestinal bleeding. Against this must be balanced the production of serum hepatitis and antihaemophilic factor inhibitors from multiple infusions of plasma products if the patients are treated conservatively.

CHAPTER 7ELECTROENCEPHALOGRAPHY IN HAEMOPHILIA
AND CHRISTMAS DISEASE

Intracranial bleeding is one of the commonest causes of death in patients with haemophilia, the mortality rate being over 70 per cent (Singer and Schneider, 1962; Alagille and Charles, 1964; Potter, 1965); most episodes of bleeding are spontaneous there being no history of trauma (Eyring et al., 1965).

It has been suggested by Denton and Gourdeau (1970) that in haemophilia subclinical bleeding into the central nervous system occurs frequently and might explain the high incidence of emotional instability and psychiatric disorders documented by Agle (1964). Evidence presented by these authors using electroencephalography suggested that abnormal tracings were present in about 50 per cent of haemophilic children who had no clinical evidence of previous intracerebral bleeding.

As this suggestion was out of keeping with our own clinical experience of a large adult haemophilic population we undertook a survey of thirty consecutive admissions to the Haemophilia Centre.

Methods and materials

Thirty patients with an age range from eleven to fifty-four years of age were studied. Twenty-five patients had classical haemophilia and five had Christmas disease. Using the clinical grading system of severity of the disease as described by Biggs and Macfarlane (1963), fifteen of our patients were severely affected, eight were moderately affected and seven had a mild defect. All patients had a neurological examination and a standard electroencephalograph (International Federation 10/20 system). Tracings were interpreted by two independent experts as normal, minor abnormality or gross abnormality.

Results

The results are shown in Table 19. Gross abnormalities were found in only two tracings. One of these patients had sustained a subarachnoid haemorrhage and the other had overt epilepsy. Three other patients had minor abnormalities in their tracings. The only patient with clinical abnormality in the central nervous system was the patient with the subarachnoid haemorrhage. Following treatment with plasma concentrates the clinical and E. E. G. abnormalities in this patient resolved.

Discussion

With modern therapy, control of haemorrhage from sites other than the brain has improved dramatically (Kerr, 1963). Intracranial haemorrhage therefore remains the major barrier to the severe haemophiliac achieving a life expectancy comparable to the general male population.

In this unselected series only three patients were unexpectedly found to have an E.E.G. abnormality. This figure (10 per cent) is within the normal expected in a hospital population (Walshe, 1958). In none of these patients was there associated clinical abnormality, history of trauma or family history of epilepsy. Our figures therefore differ markedly from these of Denton and Gourdeau (1970) who found that 50 per cent of their haemophilic patients had an E.E.G. abnormality and those of Little and Hammack (1971) who found E.E.G. abnormalities in all of six haemophilic patients examined. The reason for the discrepancy is not clear. These patients were of the same range of severity but were of different age groups. These authors surveyed a paediatric population in which the interpretation of the E.E.G. is extremely difficult. The finding of an abnormal

E. E. G. does not necessarily indicate previous intracerebral bleeding as idiopathic epilepsy has been previously recorded in haemophilia (Silverstein, 1964).

The severely affected haemophiliac is subject to intracranial haemorrhage throughout life but in individuals with disease of lesser severity, the peak incidence of bleeding appears to be in the later decades of life (Kerr, 1964(b)). The expectation therefore would be that adult patients would have a higher incidence of E. E. G. abnormality than children, if the E. E. G. abnormality was due to previous bleeding. This we have not found.

Our figures suggest that abnormalities in the electro-encephalograph are no commoner in haemophilia than in any other chronic disease state.

SECTION 3

COMPLICATIONS OF TREATMENT

"When sorrows come they come not
single spies but in battalions."

Hamlet, Act 4,
Scene 5.

CHAPTER 8 SERUM HEPATITIS

CHAPTER 9 (a) PLASMA FIBRINOGEN AND E.S.R.
 (b) BLOOD GROUP ANTIBODIES
 (c) DUPLICATION OF LDH-1

CHAPTER 10 INVESTIGATIONS OF THE EFFECT
 OF ANIMAL ANTIHAEMOPHILIC
 FACTOR ON PLATELETS

CHAPTER 8SERUM HEPATITIS IN HAEMOPHILIA AND
CHRISTMAS DISEASE(a) Introduction

Virus hepatitis is an acute inflammation of the liver and in general the definition excludes infection due to recognised viruses such as cytomegalovirus, coxsackie A and B, Herpes simplex, yellow fever and adenovirus. Two clinically similar infections are described; infectious hepatitis (epidemic jaundice) caused by virus A and serum hepatitis (homologous serum jaundice, transfusion hepatitis, inoculation jaundice) caused by virus B.

Serum hepatitis was first described in 1885 by Lürman following an outbreak of jaundice amongst a group of shipyard workers vaccinated against smallpox with glycerinated "lymph" of human origin. It was then appreciated that jaundice could be transmitted in centres where multiple injections were given such as venereal disease and diabetic clinics (MacCallum, 1943). The introduction of blood transfusion and the use of plasma products was followed by episodes of jaundice which had a long incubation

period of up to six months. This type of hepatitis could be transmitted by any procedure in which the skin or a mucous membrane was punctured by instruments contaminated with the blood of a carrier of the virus eg. needles, lancets, tattoo needles, razors, dental and surgical instruments, dialysis equipment and tissue and organ transplants. Virus hepatitis is now the most important complication of blood transfusion (Mosley, 1966; Zuckerman, 1970(a) and (b)) and in 1963 in the United States out of 1.8 million transfusions given it was estimated that the incidence of clinical hepatitis was 30,000 cases with nearly 3,600 deaths.

A major advance in the study of viral hepatitis occurred in 1964 with the discovery of the Australia (Au) antigen by Blumberg. The antigen was detected when the serum of a haemophilic patient who had received multiple transfusions was tested by immunodiffusion against that of an Australian aborigine, and was originally considered to be an example of an inherited trait (Blumberg et al., 1965 and 1967). The antigen has been found only rarely in healthy North American and European communities

and has not been found in the serum of patients suffering from various liver diseases other than hepatitis. The antigen has been shown to occur frequently in the blood of patients receiving multiple transfusions, in 20 per cent of patients with virus hepatitis and in 10 - 14 per cent of patients with leukaemia. It is also found in 1 - 20 per cent of normal people living in the tropics and in South-East Asia (Blumberg et al., 1968).

Prince (1968(a)) described the appearance of an antigen which he termed the SH antigen, in the blood of a patient during the early incubation period of post-transfusion hepatitis and this was confirmed by Okochi and Murakami (1968) who found a strong association between the Australia antigen and post-transfusion hepatitis in Japan. The SH antigen has since been shown to be identical to the Au antigen (Prince, 1968(b)). Some patients transfused blood containing AuSH antigen subsequently developed hepatitis with the antigen in their blood, while others developed antibody to it, with no evidence of hepatitis. It is known that the antigen may persist in the blood after recovery from the acute phase of hepatitis and it has been recorded over a twenty year period in an apparently healthy volunteer blood donor (Zuckerman

and Taylor, 1969) and also in the blood of half the volunteers inoculated with MS-2 strain of serum hepatitis (Giles et al., 1969).

Au-SH antigen has now been identified in all the plasma fractions used therapeutically in the treatment of haemophilia and Christmas disease (Schroeder and Mozen, 1970) and thus confirms the clinical evidence of transmission of the virus in the various fractions; eg. in fibrinogen (Anderson et al., 1960; Cronberg et al., 1963; Newcomb and Watson, 1963), in Cohn FI-O (Marder and Schulman, 1966), in cryoprecipitate (Del Duca et al., 1966; Besselaar et al., 1966; Forbes et al., 1969; Whittaker et al., 1969; Fitzpatrick et al., 1969), in glycine precipitated AHF (Brinkhous et al., 1968) and in Christmas factor concentrates (Kingdon, 1970).

As out-patient and home treatment of haemophilia becomes readily available more and more patients are receiving replacement therapy with plasma infusions with a resulting increase in exposure to hepatitis virus. This survey of an adult haemophilic population has been carried out to establish the incidence of

clinical jaundice, anicteric hepatitis and the association of the episodes with the incidence of positive reactions for Australia (SH) antigen and antibody.

Patients and methods

To obtain figures for the incidence of clinical jaundice 178 consecutive admissions to the Haemophilia Centre were questioned about jaundice and its relationship to transfusion of blood or blood products.

To determine the incidence of AuSH (HAA) antigen in the haemophilic population blood was tested by immunodiffusion techniques each time a patient was seen with a new bleeding episode and on follow up after an episode of bleeding which required blood or plasma transfusion. Assays of AuSH antigen and antibody were performed in the Regional Blood Transfusion Centre by immunoelectrophoresis. Liver function tests were also performed on these occasions. These included serum bilirubin, alkaline phosphatase, glutamic oxalo-acetic transaminase (GOT) and glutamic pyruvate transaminase (GPT) all of which were estimated by standard autoanalyser techniques. Total protein and albumin

and globulins were estimated as necessary. Bromsulphthalein retention test (45 minutes) was performed by the method of Fleck and Morrison (1970). Seventy-eight patients with haemophilia or Christmas disease were seen in this part of the survey. Out of the haemophilic population these were the patients at maximum risk as they were receiving multiple transfusions of plasma concentrates for therapeutic purposes.

Results

(b) Incidence of clinical jaundice

Twelve patients out of 128 questioned (10.1 per cent) had a past history of clinical jaundice and all the episodes had been related to transfusion of blood or plasma products in the previous six months. One patient had two episodes of jaundice within the space of one year. Seven patients were treated at home by their own practitioners and five were admitted to hospital. The main biochemical findings of this group of five are shown in Table 20. Only two of the five patients had positive AuSH antigen, none developed positive AuSH antibodies. Patient 2 has had persistence of antigen in his blood for two years now and has clinical and biochemical evidence of continuing liver cell damage.

(c) Incidence of anicteric hepatitis

In a four year period eleven out of seventy-eight patients who were repeatedly tested were found to have anicteric hepatitis. Of these eight were found on routine biochemical testing of blood samples and three had minimal symptoms. Relevant data from these patients is shown in Table 21. Two of these patients have had two separate episodes of biochemical hepatitis having totally recovered from the first attack. In only one patient was the Australia antigen found and he then developed a positive AuSH antibody. In one other patient AuSH antibody developed during the course of the hepatitis although AuSH antigen had not been found. All the patients had exposure to plasma products in the previous six months. These varied from 6 units of fresh frozen plasma to 380 units of cryoprecipitate.

(d) Incidence of AuSH antigen and antibody

In seventy-eight adult haemophiliacs who have received multiple transfusions four patients have been found with positive AuSH antigen. Two of these had clinical jaundice, one had anicteric hepatitis and one patient was found by chance to be a AuSH antigen carrier, with no previous history of hepatitis.

Ten patients (12.8 per cent) were found to have AuSH antibody when first tested. In only three of these was there evidence of previous hepatitis but all had severe haemophilia and had received multiple infusions of blood, plasma and plasma products in the past. In one patient the antibody test became negative after four months. In the other nine patients the antibody is still present. The follow up period varies from two months to two years.

Follow up of patients with evidence of hepatitis or positive Au antigen or antibody

Only thirteen patients out of a possible twenty-six with evidence of hepatitis were willing to come for B.S.P. tests and review. Four of these had abnormal B.S.P. retention at 45 minutes. In a control series of thirteen haemophilic patients of the same severity who had received an equivalent number of infusions but who had never had hepatitis and had negative AuSH antigens, four had abnormal B.S.P. retention. This would indicate an incidence of hepatic impairment of 30 per cent in haemophilic patients receiving multiple infusions.

(e) Discussion

In the history of blood transfusion it was appreciated early on that blood and blood products might transmit hepatitis (Propert, 1938; Spurling et al., 1946; Brightman and Korns, 1947; Barnet et al., 1950; James et al., 1950; Rosenthal et al., 1950; Wallace, J., 1961) and it has been shown that the incidence of hepatitis increased linearly with the number of transfusions of blood up to 6 units (Allen and Sayman, 1962). The Australia antigen has been found in all the fractions used therapeutically in haemophilia and Christmas disease (Schroeder and Mozen, 1970) but the risk of hepatitis depends on the particular fraction used; for example the risk following fibrinogen may be as much as 35 times that after whole blood (Zuckerman, 1970(a)). By virtue of the fact that effective therapy in bleeding disorders requires concentrates of the deficient factor made from pools of normal plasma the exposure of an individual patient to the virus is high.

In theory it should be possible to test every donor unit of blood for the presence of antigen and so reduce the incidence of serum hepatitis, but Alter et al. (1970) have produced evidence that this will reduce the incidence of serum hepatitis by no more than 25 per cent.

The incidence of clinical jaundice in our series was 10 per cent and this compares with a figure of 2 per cent (Rizza and Biggs, 1969), 10 per cent (Maycock et al., 1963; Newcomb and Watson, 1963), 14 per cent (Cronberg et al., 1963) and 30 per cent (Marder and Shulman, 1966). It is thought that clinical jaundice is only the tip of the iceberg and that up to 100 cases of hepatitis may occur for each overt jaundice (Hampers et al., 1964). More recent studies however suggest that only 3 - 4 anicteric cases occur for each icteric case (Mirick et al., 1965). It is probable that the thirteen episodes of anicteric hepatitis which were found fortuitously represent a gross underestimate of the incidence in the population at risk. Liver function tests are abnormal for such a short time that it would require twice weekly estimation for six months to ensure complete screening of the patients and this is impractical. In one patient hepatitis occurred despite the presence of Australia antibody in the plasma. This has been noted before by Holland et al. (1969).

In three of these patients (18.7 per cent) with evidence of hepatitis AuSH antigen was found. One of these patients (patient 2) has continued to have a positive Au-SH antigen in his

blood for two years and one has developed an AuSH antibody which has been present eighteen months. One patient (patient 14) developed an AuSH antibody without having had detectable AuSH antigen, a phenomenon reported previously by Zuckerman (1970(a)). This incidence of positive AuSH antigen is low compared with the 40 per cent of patients found to be positive by Cossart et al. (1960) and the 82 per cent by Gocke and Kavey (1969) in patients with probable serum hepatitis. It has been suggested that there are important regional variations in the incidence of AuSH antigen (Mathews and MacKay, 1970) and it has been shown to have a low incidence in the West of Scotland (Laiwah et al., 1970).

Australia antibody was found in 12.8 per cent of haemophilic patients examined. This is in keeping with the figures of 12.8 per cent found by Kunst and Rosier (1970) and of 17 per cent by Seeler and Mufson (1971). Antibody has been detected in as many as one-third of haemophilic patients (Blumberg et al., 1965; Shulman and Barker, 1969). Patients with persistence of AuSH antigen have been shown to have biochemical and histological evidence of liver impairment (Singleton et al., 1971). This was seen in both of our patients with persistence of AuSH antigen, one had hepato-cellular failure and the other abnormal retention of

B.S.P. The incidence of abnormality of B.S.P. retention is 25 per cent in patients with a previous history of hepatitis + Australia antibody and 30.6 per cent in a group of multitransfused haemophiliacs who had no history of hepatitis and who do not have AuSH antibody. There appears to be no study of the long term hepatic effects of multiple transfusions on liver histology in haemophilia although there have been sporadic case reports of death from serum hepatitis (Newcomb and Watson, 1963).

It is possible that production of higher purity AHF concentrates which contain less fibrinogen may reduce the incidence of hepatitis and also some reduction may also result from exclusion of AuSH antigen positive donors. Similarly judicious use of fibrinolytic inhibitors such as aminocaproic acid and tranexamic acid may reduce the haemostatic requirements in selected cases.

This study has shown the high incidence of serum hepatitis associated with patients receiving multiple transfusions of pooled blood products but there appears to be a low incidence of AuSH antigenaemia. We do not as yet know the relative importance of the various icterogenic viruses, but it is quite possible that virus B is not the only infective agent responsible for post-transfusion

hepatitis. It is also possible that there may be other portals of entry for infective agents other than transfusion.

CHAPTER 9CHANGES IN PLASMA FIBRINOGEN AND
ERYTHROCYTE SEDIMENTATION AFTER
TREATMENT WITH CRYOPRECIPITATE(a) Introduction

The preparation of cryoprecipitate involves the cold precipitation of various factors in blood plasma. These consist mainly of fibrinogen and antihaemophilic factor with smaller amounts of the other globulins, albumin and some coagulation factors (Table 22). The amount of fibrinogen varies from batch to batch but average cryoprecipitate contains about 40 - 50 per cent of the fibrinogen from the starting plasma. Brown et al. (1967) found the levels of clottable protein in cryoprecipitate to be eleven times that of the starting plasma but we have found it to be only 2 - 3 times that of plasma (Forbes et al., 1969). As the half life of infused fibrinogen is between 4 - 6 days (Madden and Gould, 1952; Zetterquist et al., 1964) compared with the half life of infused antihaemophilic factor of about ten hours (Douglas, 1958; Biggs and Denson, 1963; Abildgaard et al., 1964),

the result of cryoprecipitate therapy is a rise in the plasma level of fibrinogen. This may reach massive levels, the highest reported being 2,500 mg/100 ml (Marder and Shulman, 1966). We record here the changes in plasma fibrinogen associated with cryoprecipitate therapy and the effect on the sedimentation of red blood cells.

Fibrinogen and Erythrocyte Sedimentation Rate (ESR)

Fibrinogen and ESR were measured before and after therapeutic infusion of various amounts of cryoprecipitate. The results are shown in fig. 29 and the differences are highly significant in both instances ($p < 0.001$). Fig. 30 shows the relationship between the ESR and the fibrinogen levels in a series of forty-three patients both treated and untreated ($r = 0.3255$). Fig. 31 shows the changes in fibrinogen, ESR and AHF levels which occurred in one representative patient.

Discussion

The production of very high levels of plasma fibrinogen by infusion of plasma products is usually thought to be a benign consequence of replacement therapy with only the minor problems of

rouleaux formation and occasional difficulty in crossmatching blood (Marder and Shulman, 1966; Bloom et al., 1968). There are however occasional reports of mild haemolysis in the recipient (MacMillan et al., 1961; Le Quesne et al., 1967) and a suggestion that activation of the fibrinolytic enzyme system may occur (Shanbrom, 1968). The evaluation of elevated fibrinogen levels in the aetiology of haemolysis is extremely difficult as these patients are usually the most severely ill, have had major surgery, have bacterial infections and usually have had whole blood transfusions. It has been shown that glycine precipitated preparations of AHF contain iso-antibodies to the main blood groups (Abildgaard et al., 1966) but we have been unable to show this in cryoprecipitate (Forbes et al., 1969). In a recent case report we described a haemophilic boy who developed acute intravascular haemolysis when his plasma fibrinogen was 1000 mg per cent (Forbes et al., 1972). The picture in this patient was complicated by concomitant therapy with cephalixin, and prior therapy with ampicillin. In retrospect it is not possible to be certain of the part played by these various factors. The full report of this patient is in the Appendix 4. It is unusual, in that

the plasma level of cephalixin was extremely low and other factors, such as high blood urea and low albumin were absent.

(b) Blood group antibodies

It is well known that patients receiving multiple transfusions of blood develop antibodies against blood group antigens.

Eleven (8.6 per cent) out of 128 haemophilic patients have been found with blood group antibodies (Table 23). The commonest antibody was to the Rh system (eight patients).

Three patients had antibodies to the Kell group. All the patients had a moderate or severe degree of haemophilia or Christmas disease and all had received many transfusions of whole blood, plasma or cryoprecipitate and because of this it is not certain how isoimmunisation came about. In one patient anti-D antibody appeared for the first time after the patient had received a course of cryoprecipitate alone. This is the first documented case of accidental production of anti-D after cryoprecipitate therapy and presumably represents carry over of red cells with D antigen during preparation. Minute amounts of red cells or red cell stroma can produce Rh-antibodies (Waller, 1949; Wiener, 1949) and we have estimated that any single pack of cryoprecipitate may

contain 0.1 ml of trapped cells. This individual received 190 packs of cryoprecipitate which may have contained up to 19 ml of red cells. No attempt has been made in the past to give therapeutic materials according to the blood groups of the donors or of the recipient as this had always been considered unnecessary.

The importance of knowing the iso-antibody status of these patients is the avoidance of transfusion of Rh positive or Kell positive blood which might produce acute intravascular haemolysis (Wiener and Sonn-Gordon, 1947).

(c) Duplication of LDH-1 in a patient receiving
multiple transfusions

Introduction

The existence of multiple molecular forms of lactic dehydrogenase is well documented and five iso-enzymes have been demonstrated (Vesell and Bearn, 1957; Wieland and Phleiderer, 1957). Differences in the concentration of the individual iso-enzymes have diagnostic significance in diseases of heart (Cohen et al., 1964; Starkweather et al., 1966), liver (Weime and Van Maercke, 1961), striated muscle (Emery, 1965)

and red blood cells (Hess and Walter, 1961). Fujimoto et al. (1968) have shown additional bands between LDH-1 and -2 and between LDH-2 and -3 in a patient with oesophageal carcinoma with hepatic secondary deposits. They concluded that the bands were produced by the tumour cells and represented an altered form of the M subunit, which produced a set of hybrids with the normal H subunit. We report here the unique observation of duplication of LDH-1 after multiple transfusions of plasma products. This is the first demonstration of heterogeneity of LDH iso-enzymes in the population.

Case history

The patient was a severely affected haemophiliac aged 14 years. He sustained a rupture of the left ureter with a massive retroperitoneal haematoma as a result of a crushing injury. Surgery was undertaken under cover of cryoglobulin precipitate, which is a crude concentrate of antihæmophilic factor made by the cold precipitation of human plasma (Pool et al., 1964). This therapy was continued for 30 days to permit healing of the wound and resorption of the haematoma. Cryoglobulin precipitate from 1,500 donors was used in this patient.

Methods

Electrophoretic separation of lactate dehydrogenase (LDH; L-lactate : NAD oxidoreductase, EC 1.1.1.27) iso-enzymes was carried out on cellulose acetate and zones of activity were made visible by incubating the strips in contact with (2 g/dl) agar, Tris-buffered to pH 8.4 and incorporating, per litre, 100 mmol of lactate, 1.5 mmol of NAD, 66 μ mol of phenazine methosulphate, 500 μ mol of thiazolyl tetrazolium and 6 μ mol of semicarbazide hydrochloride.

On the fifteenth day after the patient's admission it was noted that the fastest anodal migrating iso-enzyme (LDH-1) showed two distinct bands. This splitting of the LDH-1 zone was demonstrable until the nineteenth day after admission and was also shown when hydroxybutyrate or glycerate was substituted for lactate. No formazan dye was produced in this position in the specimens affected. Simultaneous electropherograms of sera obtained on the fourteenth, fifteenth, nineteenth, and twentieth days are shown in Fig. 32.

During this period the plasma LDH levels were between 700-1000 U/litre.

Following a suggestion by Professor J. H. Wilkinson, we progressively decreased the quantity of serum subjected to electrophoresis but a similar splitting of the LDH-2 band, the most prominent, could not be demonstrated.

It is likely that the additional band was not an artefact, but represented an abnormal H subunit present in one more of the transfused plasmas and suggests heterogeneity of the LDH₁ iso-enzyme molecule in the population. This is the first demonstration of this phenomenon.

CHAPTER 10AGGREGATION OF HUMAN PLATELETS BY
COMMERCIAL PREPARATIONS OF BOVINE
AND PORCINE ANTIHAEMOPHILIC FACTOR(a) Introduction

Antihaemophilic factor (AHF) prepared from bovine and porcine plasma is of proven value in the management of haemorrhage in haemophilia (Biggs, 1967). It has been effectively used in human patients (Macfarlane et al., 1954; Macfarlane et al., 1957; Macfarlane and Biggs, 1959; Biggs, 1960; Rizza and Biggs, 1969), in haemophilic dogs (Sharp and Dike, 1964) and in a haemophilic horse (Nossel et al., 1962). Bovine AHF at concentrations of 0.5 mg/ml produces platelet aggregation both in vitro (Sharp and Bidwell, 1957) and invariably in vivo (Macfarlane and Biggs, 1959). Thrombocytopenia was also recorded in the haemophilic horse treated with bovine AHF (Nossel et al., 1962). The effect of porcine AHF preparations in vivo and in vitro is not so well defined. Sharp and Bidwell (1957) showed no effect on human platelets in vitro when the porcine preparation was used

at 0.5 mg/ml but when the concentration was raised to 5 mg/ml platelet aggregation could be produced. Occasional cases of transient thrombocytopenia have been recorded in human haemophilic patients during therapeutic administration of porcine AHF (Macfarlane et al., 1957; Rizza and Biggs, 1969).

Materials and methods

Collection of blood: All samples were collected by clean venepuncture from healthy human donors using plastic syringes. Nine volumes of blood was added to one volume of 3.8 per cent sodium citrate or 2 per cent ethylenediaminetetracetic acid. All glassware was siliconised using "Siliclad" (Clay Adams Inc., New Jersey, U.S.A.).

Platelet rich plasma: was prepared from anticoagulated whole blood by centrifugation at 600 g for five minutes at room temperature.

Porcine and bovine antiphaemophilic factor: preparations were purchased from S. Maw & Son, Barnet, England. The contents of each ampoule was dissolved in 50 ml of normal saline and stored at -20°C . The average weight of dry material per vial of bovine AHF was 577 mg and of porcine material was 407 mg.

The dilutions referred to in the text were prepared from this stock solution. The fibrinogen content of these materials was (bovine) 576 mg/100 ml and (porcine) 350 mg/100 ml.

The methods used to prepare these animal products are essentially those described by Bidwell (1955 (a) and (b)) and depend on fractionation with potassium phosphate and sodium citrate. This results in a preparation of AHF between 100 - 400 fold concentrated with respect to the starting plasma.

Adenosine diphosphate (ADP): Sigma Chemical Company, St. Louis, Missouri. A stock solution of 100 μ g ADP per ml was prepared and kept at -20°C . Dilutions referred to in the text were made from this.

Collagen: was extracted with saline from human Achilles tendon.

Animal plasmas (Colorado, U. S. A.): were purchased from the Colorado Serum Company and were kept frozen at -20°C .

Plasma from a range of primates was obtained from the Laboratory for Experimental Medicine and Surgery in Primates (L. E. M. S. I. P.) New York University Medical Center, New York. All samples were obtained deep frozen in 4 per cent sodium citrate.

Plasma from the four animal species which produced maximum platelet aggregation was clotted by addition of 0.025 M CaCl_2 and the serum incubated at 37°C for three hours to remove any residual thrombin. Platelet aggregation of the starting plasma was then compared with the incubated serum.

Adenosine: (Sigma Chemical Company Limited, London) was made up in normal saline and used at a final concentration of 50 $\mu\text{g}/\text{ml}$ of platelet rich plasma.

Celite 512: (Johns-Manville Company, Celite Division, New York, N. Y.).

Sephadex G-200 and Sepharose 4B: (Pharmacia, Fine Chemicals, Uppsala, Sweden) were expanded in barbital-saline buffer (0.15 M, pH 7.3).

Plasmin: (A.B. Kabi Pharmaceuticals, London) was dissolved in normal saline immediately before use to the required concentration.

Thrombin: (Parke, Davis and Company, Detroit, Michigan, U. S. A.) was diluted in barbital saline buffer as required.

Agarose: (BDH Chemicals Limited, Poole, England) was made up to 0.9 per cent in barbital buffer pH 8.4, 0.05 M.

Rabbit anti-human fibrinogen antiserum: was purchased from Behringwerke A. G.

Bovine and porcine fibrinogen: Koch Light Laboratories Colnbrook, England, were dissolved, as required, in distilled water to give the required concentration.

Clinical study: Three haemophilic patients were given porcine AHF therapeutically. Two underwent major surgery and one, with an AHF inhibitor, was bleeding from the gastrointestinal tract. Standard dosage schedules as recommended by the manufacturers were given except in the third patient in whom twice the standard dose was eventually given in an attempt to overcome the inhibitor and provide a haemostatic level of AHF.

Platelet aggregation: was measured by the turbidimetric method of Born (1962). The apparatus used in this study consisted of an E. E. L. titrator connection to a galvanometer (E. E. L. type 20, Evans Electro-selenium Limited, England). A perspex cuvette was fitted on to a titrator above a magnetic stirrer and in the light path from the photoelectric cell. Platelet rich plasma, 2 ml amounts, was added to the cuvette together with a small stirring rod. When the stirring rod was

rotating at a uniform speed, the optical density reading of the plasma sample on the galvanometer scale was adjusted to an arbitrary value of 0.600. The plasma was agitated for 30 seconds to ensure that no change in optical density occurred before addition of 0.1 ml aliquots of aggregating reagent. Changes in light transmission, at a wavelength of 492 m μ , were recorded at 30 second intervals over a period of ten minutes from the addition of the test material. Control samples were handled in an identical fashion except that normal saline replaced the test solution.

Platelet adhesiveness: was measured by the glass bead column method as described by Hellem (1960), and modified by Hirsh et al. (1966). The glass bead column was made by filling a length of vinyl tubing (NT/13, Portland Plastics, Kent) with 2.5 g Ballotini glass beads (0.57 mm in diameter) to give a column 6 cm in length. The glass beads were held in the column by a filter of fine nylon gauze fitted at each end of the column. 2 ml of platelet rich plasma were incubated for 10 minutes at 37°C with 0.1 ml aliquots of porcine or bovine AHF then drawn into a graduated plastic syringe which was then fitted to an electrically operated mechanical pump. The plasma was pushed through the column of beads at a constant rate. The apparatus used in

the study gave a mean contact time between plasma and glass of 30 ± 1 seconds. Platelet counts were performed on the plasma samples before and after passage through the column. The difference between the two counts was expressed as a percentage of the initial platelet count and this value taken as an index of platelet adhesiveness in the sample. This was repeated in seven different plasma samples.

Chandler tube technique: As described by Chandler (1958) and modified by Cunningham et al. (1965). The transparent vinyl tubing, 12.3 mm bore, 71 cm in length was made into loops by means of a nylon adaptor. The loops were washed in cold water and rinsed in 0.9 per cent sodium chloride. Citrated platelet rich plasma, 5 ml aliquots, was added to the two loops and the volume made up to 15 ml with saline. AHF solution or saline (0.25 ml) was added to the system and the loops rotated on the turntable of a blood cell suspension mixer (Matburn Limited, London) at 28.5 rpm in a glass fronted incubator at 37°C . The platelet aggregation was assessed by the length of time taken for the appearance of a "snowstorm" effect. This was repeated on seven different plasma samples.

Preparation of rabbit antibody against partially purified porcine

AHF: Partially purified porcine AHF prepared by gel filtration on Sepharose 4B was concentrated by pressure dialysis on an Amicon using a PM30 membrane. Two ml of concentrated AHF, containing approximately 20,000 units of AHF activity and 5 mg of protein was mixed with 2 ml of Freund's complete adjuvant and injected intramuscularly into a white New Zealand rabbit. The injection of antigen was repeated at three weeks, then one week later and the animal was bled ten days after the last injection. The serum was allowed to clot overnight then heated at 56°C for thirty minutes. To make the antibody monospecific for AHF, equal volumes of antibody were incubated for one hour with purified porcine fibrinogen prepared by gel filtration. The fibrinogen was removed by heating at 56°C for thirty minutes and the antibody concentrated by absorbing the water with Lyphogel (Gelman Instrument Company).

Platelet counts: were performed by the method of Dacie (1956) using formol citrate as the diluting fluid.

Antihaemophilic factor: was assayed by the method of Margolis (1958) as modified by Breckenridge and Ratnoff (1962).

Dialyses: were performed at 4°C in cellophane casings (Visking Corporation, Chicago, Illinois).

Ultracentrifugation: was carried out in a Beckman Model L ultracentrifuge, the samples being spun at 60,000 g for twenty-four hours.

Acrylamide gel disc electrophoresis: was performed at pH 9.5 as described by Davis (1964) using a standard 7 per cent gel. The gels were stained with 0.5 per cent aniline black in 7 per cent acetic acid.

Immunodiffusion: was carried out by the method of Ouchterlony (1962) in 0.9 per cent agarose in barbital buffer, pH 8.4, 0.05 M.

Protein: protein concentration was determined by the method of Lowry et al. (1951), or estimated more crudely by absorption at 280 mμ in a Beckman Model DU spectrophotometer.

Gel filtration of human platelets: was carried out by the method of Tangen et al. (1971). Platelet rich plasma (p.r.p.), prepared as described previously from human blood collected in acid-citrate dextrose anticoagulant, was filtered on a 1.5 x 30 cm column of Sepharose 2B (Pharmacia Fine Chemicals Inc.).

The volume of platelet rich plasma applied to the column varied from 0.5 - 1.5 ml and the eluting buffer was 0.145 M NaCl, pH 7.4 with 0.005 M Trizina base containing 5×10^{-5} M Ca Cl_2 and 0.1 per cent glucose. Platelets prepared by this method are free of non-absorbed plasma proteins and aggregate with ADP and collagen.

Results

(b) Effect of bovine and porcine AHF on human platelets

1) Platelet aggregation in vivo by porcine AHF: Infusion of porcine AHF into haemophilic patients resulted invariably in thrombocytopenia. In the first two patients (fig. 33) thrombocytopenia was transient and the platelet count returned to normal within twenty-four hours. However in patient 3 (fig. 34) severe, prolonged, thrombocytopenia was produced with exacerbation of gastrointestinal bleeding and a rise in blood transfusion requirements. No rise in AHF levels could be detected in this patient.

2) Platelet aggregation in vitro by bovine and porcine AHF

preparations: Bovine and porcine AHF preparations are potent platelet aggregating agents in vitro (fig. 35). When either was added to human platelet rich plasma, in concentrations

equivalent to those obtained during therapeutic administration, irreversible platelet aggregation occurred within two minutes. At lower concentrations the effect was less marked and aggregation became reversible. On a weight for weight basis the bovine material was approximately four times more potent, in producing platelet aggregation than the porcine material.

Platelet aggregation was produced by samples from ten separate batches of bovine and porcine AHF preparations.

3) In vitro effect of various animal plasmas on aggregation of human platelets: The results are shown in fig. 36. Plasma from goat, sheep, pig, cow and horse produced aggregation of human platelets in vitro. Plasma from a range of primates (chimp, rhesus monkey, black ape, baboon, gibbon and squirrel monkey) produced very slight, reversible aggregation and plasma from a wide range of animals (dog, cat, rat, opossum, rabbit, hamster, guinea pig, turkey, pigeon, chicken, paddlefish and lobster) produced no aggregation.

Comparison of the aggregating action of four animal plasmas with their sera is shown in fig. 37. In all four cases

clotting results in a marked diminution of aggregating ability but this did not entirely disappear.

4) Platelet adhesiveness by the method of Hellem: The mean adhesiveness of the seven control samples using platelet rich plasma is 12 per cent \pm 8 per cent and the seven test samples 79 per cent \pm 4 per cent. This difference is highly significant ($t = 22$, $p < 0.001$).

5) Effect on the Chandler tube system: The average time for the appearance of the "snowstorm" in the seven tests was 141 seconds with a standard deviation of seventeen seconds. The "snowstorm" consisted of aggregates of 50 - 100 platelets. No "snowstorm" appeared in the control tubes and no fibrin formation was seen in either tube.

(c) Physical and chemical properties of the platelet aggregating agent

1) To determine if the aggregating agent was a protein: An equal volume of 10 per cent trichloroacetic acid (TCA) was added to the AHF preparations (one-tenth volume) and the resulting precipitate was centrifuged at 2000 rpm for ten minutes. A

control tube was set up in which an equal volume of saline was added to the AHF preparation. Aliquots (0.1 ml) were then added to platelet rich plasma in the aggregometer. This results in complete loss of aggregating activity. TCA in this concentration had no effect on platelet aggregation.

2) Dialyses: Dialyses of solutions of animal AHF were performed at 4°C in cellophane casings (Visking Corporation, Chicago, Illinois) using at least forty times the sample volume of barbital saline buffer. After twenty-four hours no significant change could be detected in aggregating activity.

3) Absorption: Absorption was performed with calcium phosphate or celite 512, 10 mg/ml of AHF preparation at room temperature. The absorbent was removed by centrifugation and eluted with 10 per cent sodium chloride. The eluate was dialysed overnight against barbital-saline buffer. Aggregating activity of the supernatant and the eluate was tested and the activity was shown to remain in the supernatant. No activity was found in the eluate from the calcium phosphate or celite.

4) Effect of temperature: Aliquots of the AHF preparations were kept for ten minutes in a waterbath at 40°C, 50°C, 60°C, 70°C. Any precipitate which formed was removed by centrifugation at 800 g for ten minutes. Samples of stock AHF solutions were also kept at -20°C for two years and at room temperature for one week. The results are shown in fig. 38 (a) and (b). These results indicate progressive destruction of the platelet aggregating factor as the temperature is raised, with complete destruction of aggregating activity when the preparations were kept at 70°C for ten minutes.

The aggregating factor is stable when kept at room temperature for a week and also at -20°C for two years.

5) Ultracentrifugation: of the crude animal AHF was carried out in a Beckman Model L ultracentrifuge, the samples being spun at 60,000 g for twenty-four hours. This had no effect on the aggregating activity which was present to the same degree at the top and bottom of the tube.

6) Inhibitors of platelet aggregation: The effect of collecting the platelet rich plasma in 2 per cent ethylenedramine tetracetic acid was determined and also the effect of preincubation of

citrated platelet rich plasma at 37°C with adenosine and heparin. Addition of ethylene diamine tetracetic acid (E. D. T. A.) to the platelet rich plasma resulted in inhibition of aggregation.

Similarly preincubation of platelet rich plasma with adenosine at a concentration of 50 µg/ml of plasma resulted in inhibition of aggregation, as did dipyridamole ("Persantin").

Heparin produced no inhibition of aggregation even in concentrations of 25 u/ml of plasma.

7) Effect of ingestion of acetylsalicylic acid ("aspirin", a. s. a.)

on bovine and porcine AHF induced platelet aggregation: Four volunteers were given 1.2 g of acetylsalicylic acid after blood had been removed for assessment of platelet aggregation by animal AHF. Samples were taken two hours afterwards and platelet aggregation retested. The aggregating agents tested were collagen and crude bovine and crude porcine AHF preparations at a final concentration of 2.5 mg/ml of plasma.

In all four experiments similar findings were made. Collagen induced aggregation was totally abolished and the secondary phase of platelet aggregation seen with the animal materials was abolished.

The results of one of these four experiments is shown in fig. 39 (a) and (b).

(d) Purification of the platelet aggregating agents

Gel filtration of crude porcine or bovine antihæmophilic factor

by Sepharose 4B. 250 mg of crude porcine or bovine AHF were dissolved in 3 ml of barbital-saline buffer then sufficient sucrose was added to give a concentration of 10 per cent. This was applied to a column of Sepharose 4B, measuring 2.5 x 35 cm, equilibrated in barbital-saline buffer. The protein was washed through the column with barbital-saline buffer and the effluent collected in 3 ml aliquots. This was then examined for protein concentration, aggregating activity, antihæmophilic factor activity, and for the presence of fibrinogen by addition of thrombin (10 μ /ml) and calcium chloride, 0.025 M.

The results are shown in figs. 40 (a) and (b).

Two protein peaks were easily and consistently separated. The major peak contained fibrinogen as evidenced by the appearance of fibrin on the addition of thrombin and calcium. These tubes contained no platelet aggregating activity or AHF activity.

Polyacrilamide disc gel electrophoresis of this peak shows a single line (fig. 41, left).

The smaller protein peak, preceding the fibrinogen peak, contains all the platelet aggregating activity and the AHF activity (figs. 42 (a) and (b)). No fibrin could be detected in the peak tube on addition of thrombin but polyacrilamide disc gel electrophoresis of a concentrate ($\times 100$) showed a line in the fibrinogen position (fig. 41, right) but no other line suggesting the presence of AHF was found. This is probably due to the inability of the large AHF molecule to enter the separating gel, a finding reported previously for human AHF by Kass et al. (1969). Injection of this peak into a rabbit confirms the presence of an impurity which can be readily removed by absorption of the antibody with purified fibrinogen (fig. 43).

The aggregating activity in the peak tube was about one-third that of the starting material as was the level of AHF activity. This represents a 100 fold increase in purity of the aggregating protein.

Does the purified fraction produce the same pattern of platelet

aggregation as the crude AHF ?

Platelet aggregation was

tested using both crude porcine AHF and the purified fraction.

A volume of 0.1 ml of aggregating agent was added to 2 ml of platelet rich plasma.

The results of this experiment are shown in fig. 44.

A similar pattern of aggregation is produced by the crude and the purified material. At a critical concentration a biphasic reaction is seen in both preparations.

Aggregation of human platelets at 37°C and 24°C (room tem-

perature)

Purified bovine AHF (200 µg/ml) was added to

stirred platelet rich human plasma both at 37°C and at 24°C

(room temperature) in an attempt to see if the pattern of aggregation of platelets was different.

The results are shown in fig. 45. Similar patterns of irreversible platelet aggregation are found at an AHF concentration of 10 µg/ml but at 5 µg/ml the biphasic pattern is lost when aggregation is carried out at room temperature. Similarly at 2 µg/ml slight reversible aggregation occurs at 37°C but not at 24°C.

Association of the platelet aggregating activity with antihæmophilic factor

1) Is the aggregating activity associated with AHF activity?

Samples of purified porcine and bovine AHF were diluted with barbital-saline buffer to provide a concentration of aggregating activity which produced rapid, irreversible aggregation of platelets in human platelet rich plasma. This material was then incubated with one-tenth volume of plasma from a hæmophiliac with a potent AHF inhibitor and as a control, one-tenth volume of plasma from a hæmophiliac without an inhibitor. Platelet aggregation and AHF assays were measured at zero time and after incubation for sixty minutes.

In two separate experiments the antihæmophilic factor activity of the purified porcine or bovine fraction was totally destroyed without any change in the platelet aggregating activity. The starting level of AHF in one experiment was 2.80 units/ml and in the other 1.0 units per ml. The results of one experiment are shown in fig. 46.

2) Association of aggregating activity with the antihaemophilic factor molecule

Partially purified porcine AHF was prepared by gel filtration as already described. A fraction was chosen which produced rapid irreversible platelet aggregation. Equal volumes of this fraction were incubated with one-tenth volume of the monospecific anti-AHF antibody or control rabbit serum for thirty minutes at room temperature. The tubes were then spun at 3000 g for thirty minutes and tested for platelet aggregating activity. AHF activity was assayed at the beginning and end of the incubation period.

The results are shown in fig. 47. Incubation of the partially purified AHF with a monospecific antibody to AHF results in total removal of the aggregating activity. No apparent change was noted in control tube. Addition of the antibody also resulted in total loss of AHF activity. The assay level of AHF in the control tube was unchanged at 2.3 u/ml and in the antibody tube was 0.01 u/ml.

3) Association of aggregating activity with fibrinogen

In a similar experiment to that described above the use of a monospecific antifibrinogen antibody had no effect on platelet aggregation.

(e) Aggregation of human platelets by commercial
bovine and porcine fibrinogen preparations

Commercial preparations of both bovine and porcine fibrinogen were obtained from Koch-Light Laboratories. Both preparations were made up in saline to concentrations of 400 mg per cent and 800 mg per cent.

At concentrations of both 4 mg/ml and 8 mg/ml bovine fibrinogen produced platelet aggregation. Porcine fibrinogen at a concentration of 8 μ g/ml produced only slight aggregation (fig. 48).

Gel filtration of bovine and porcine fibrinogen preparations and

Sephacrose 4B 250 mg of commercial bovine or porcine fibrinogen in 5 ml of saline were filtered through Sepharose 4B equilibrated with barbital-saline. Aliquots of 5 ml were collected and analysed for protein, fibrinogen, antihaemophilic factor and aggregating activity.

As shown in fig. 49 (a) and (b) a pattern similar to that produced by filtration of commercial animal AHF was obtained. There were at least two protein peaks. The first peak contained all the platelet aggregating activity as well as the AHF activity.

No thrombin clottable material was present in this fraction.

The second, larger peak contained fibrinogen as evidenced by the production of fibrin by thrombin. No platelet aggregating activity or significant levels of AHF were found in this fraction.

To determine if gel-filtered platelets were aggregated by purified bovine and porcine AHF Gel filtered platelets (2 ml)

were substituted for platelet rich plasma in the Born system and 0.1 ml of the purified AHF fractions added. Irreversible aggregation of platelets occurred at the concentrations shown in fig. 50.

Effect of incubation of human plasmin with purified AHF preparations Preparations of purified bovine AHF (200 $\mu\text{g}/\text{ml}$) and purified porcine AHF (350 $\mu\text{g}/\text{ml}$) were incubated with purified human plasmin 1 u/ml and 0.25 u/ml respectively. Appropriate controls were set up with normal saline. Samples were assayed for AHF activity and aggregating activity at various time intervals as shown in figs. 51 (a) and (b). In the experiment using bovine AHF the preincubation sample of AHF activity assayed at 430 per cent and produced rapid irreversible aggregation. By fifteen minutes no activity could be detected using the assay; platelet aggregating activity had been substantially reduced.

In the second experiment using porcine AHF with plasmin at a lower concentration (0.25 u/ml) the starting level of AHF activity was 340 per cent but by fifteen minutes of incubation no activity was detectable. However it was only after 120 minutes of incubation that a significant reduction in platelet aggregation was obtained. Total loss of aggregating activity was found after sixteen hours of incubation.

To determine if animal platelets are aggregated by porcine or

bovine AHF Platelet rich plasma was obtained from a wide range of animals by drawing the blood directly into one-tenth volume of sodium citrate (0.14 M). Species examined included, pig, horse, cow, goat, rabbit, mouse, rat, dog, and guinea pig.

No aggregation of platelets of any species except the guinea pig was found with either bovine or porcine AHF.

The results of aggregation of guinea pig platelets by crude bovine and porcine AHF is shown in figs. 52 (a) and (b).

(f) Discussion

Both bovine and porcine AHF preparations cause aggregation of human platelets in vivo and in vitro. This action is

intrinsic to the native plasma from which the AHF is prepared and is also found in the plasma of goat, sheep and horse. Aggregating activity is found at low levels in primates but is absent from many other mammals and all fish and fowl tested.

The aggregating activity is precipitated by ten per cent trichloroacetic acid and is inactivated by heating at 60°C for ten minutes. It is not absorbed by celite 512 or calcium phosphate and is therefore not related to the contact or vitamin K dependent coagulation factors. Aggregation was not inhibited by heparin which inhibits thrombin induced platelet aggregation (Clayton and Cross, 1963).

The pattern of platelet aggregation is temperature dependent and is biphasic, aggregation starting on addition of the crude AHF preparation without the delay seen with collagen (Zucker and Borelli, 1962; Hovig, 1963). Identical patterns of platelet aggregation are produced by crude and partially purified AHF. The reaction is dependent on calcium ions and is blocked by preincubation of the platelet rich plasma with adenosine, a competitive inhibitor of endogenous ADP release. The second phase of aggregation can be blocked by prior ingestion of acetyl-

salicylic acid which is thought to produce inhibition of release of endogenous platelet ADP (Zucker and Peterson, 1968). The aggregating activity is also destroyed by incubation with plasmin.

Gel filtration of the crude AHF preparations shows that two major protein fractions are present. The largest fraction is fibrinogen which gives a single line on polyacrilamide disc gel electrophoresis. The smaller of the two fractions contains coincidental peaks of AHF and aggregating activity. This material is not pure and disc gel electrophoresis of a concentrated sample yielded a line in the same position as fibrinogen. Antibodies produced against this fraction contained two lines on immunodiffusion plates.

The possibility existed that the aggregating activity was either AHF or a high molecular weight polymer of fibrinogen. In an attempt to destroy AHF activity, samples of the purified animal AHF were incubated with a potent inhibitor obtained from a patient with haemophilia. Despite total loss of AHF activity as measured in the coagulation assay system no changes resulted in platelet aggregation. In unpublished experiments we have shown that these inhibitors do not alter the level of AHF like antigen

in plasma (Forbes and Prentice, 1972). However addition of a potent monospecific antibody against AHF resulted in rapid loss of platelet aggregating and AHF activity. Addition of an antifibrinogen antibody had no effect on aggregation of platelets or AHF activity. This suggests that the aggregating activity is due to a property of the AHF molecule rather than the active site or sites which participate in the coagulation cascade.

The disappearance of the aggregating agent from animal plasmas when they are clotted, or from crude porcine and bovine AHF on the addition of small amounts of thrombin probably reflects destruction of the AHF molecule during the clotting process or loss from absorption onto fibrin. AHF activity has not been detected in serum.

The finding of agglutinins to human platelets in animal plasmas is not new. Le Roy et al. (1960), Brinkhous et al. (1965) and Mason and Read (1967) described an activity present in the fibrinogen fraction of various animal species which they called thrombocyte agglutinating activity (TA_g). Solum (1968) showed that highly purified bovine platelet fibrinogen aggregated

human platelets, however, some of the aggregating activity remained despite the removal of all thrombin clottable material.

Bovine fibrinogen prepared commercially has been used as a platelet aggregating agent by Caen et al. (1966 (a) and (b)), in patients with thrombasthenia whose platelets were not aggregated by ADP, adrenaline, noradrenaline, 5-hydroxytryptamine, thrombin, purified collagen, crude connective tissue or human fibrinogen. Bovine fibrinogen at a concentration of 1.5 mg/ml produced aggregation in five of the six cases tested. These authors also showed that the aggregating activity could be destroyed on incubation with plasmin and the reaction did not consume complement. This reagent is now used to define certain platelet abnormalities eg. Thrombasthenia, Bernard-Soulier Giant Platelet Syndrome (Council on Thrombosis, A.H.A., 1972).

Gel filtration on Sepharose 4B of commercially available preparations of bovine and porcine fibrinogen shows that there are two protein peaks which are identical with those obtained after gel filtration of commercial AHF preparations and it has been shown that the platelet aggregating material is contained within the AHF peak and is not related to the fibrinogen fraction.

The mechanism by which partially purified animal AHF aggregate human platelets is unknown. As the reaction is bi-phasic and is blocked by prior ingestion of acetyl-salicylic acid this would suggest active transport of the material through the platelet membrane. Falcao et al. (1967) using electron microscopy claimed to have shown the presence of a cementing substance between the platelets and no change in the external membranes of platelets aggregated by bovine fibrinogen preparations. We have been unable to confirm this using purified AHF and gel-filtered platelets. It is possible that the appearances described by these authors were a result of non-specific trapping of plasma protein.

Infusion of animal AHF preparations into haemophilic patients gives rise to thrombocytopenia which is transient and is related to the dose given. Thrombocytopenia is probably due to trapping of large platelet aggregates in the capillaries a phenomenon which is similar to the retention of platelet aggregates in the Hellem glass bead columns. Bleeding as a result of the low platelet count has been occasionally documented (Biggs and Macfarlane, 1959; Hall et al., 1962; Rizza and Biggs, 1969),

despite a normal or high level of AHF. In one of our patients (case 3) there was no rise in the plasma level of AHF due to inactivation by inhibitor but there was evidence of increased gastrointestinal blood loss with a rise in blood transfusion requirements and a fall in haemoglobin which corresponded to the thrombocytopenic episode.

All three of the patients experienced pain along the line of the vein through which the AHF solution was administered and also suffered thrombosis in the veins at the infusion site. This thrombogenic effect has been recorded previously (Biggs, 1960), and may be due to adherence of platelet aggregates to the endothelium of the vein with production of platelet thrombi. This phenomenon has been observed by Berman and Fulton (1961) and Mustard et al. (1962) while infusing other platelet aggregating agents. Such platelet thrombi may stimulate formation of fibrin with resultant occlusion of the vessel.

Examination of a blood film in one of our patients during infusion of porcine AHF showed multiple platelet aggregates consisting of 30 - 50 platelets, measuring 100 - 200 μ in diameter.

Such platelet aggregates could have pathological consequences but this has not been proven in man. However, in experimental situations, a similar picture can be produced by infusion of adenosine diphosphate intravenously. Transient thrombocytopenia has been produced by this means in humans (Davey and Lander, 1964), rats (Nordoy and Chandler, 1964), cats (Born and Cross, 1963), swine (Mustard et al., 1964; Murphy et al., 1964) and in rabbits (Regoli and Clark, 1963). In experimental animals, platelet aggregates induced by ADP, can be seen blocking capillaries in the lung (Nordoy and Chandler, 1964) where they may produce irreversible tissue changes even though the platelet thrombus itself is transient. Mustard et al. (1964) produced myocardial infarction in swine by infusion of ADP; and in the rat, infusion of ADP may result in respiratory arrest with platelet thrombi in the pulmonary capillaries, a reaction that can be produced under experimental conditions by the injection of other embolising particles (De Takats, 1939). In the rabbit, if platelet aggregates are formed within the renal circulation, focal acute glomerulonephritis is produced with areas of cortical

necrosis (Glynn et al., 1966). In these experiments fibrin thrombi were found in the renal arteries and glomerular capillaries twenty-four hours after ADP infusion. This finding was interpreted by the authors as indicating that the fibrin thrombi were secondary to the platelet aggregates occluding blood flow.

In the original toxicity studies by Sharp and Bidwell (1957) injection of AHF preparations into rabbits produced no histological changes of note. This was probably because rabbit platelets are not aggregated by bovine or porcine AHF preparations. This is in keeping with the finding of Le Roy et al. (1960) that there is marked interspecies differences to a range of aggregating agents. A wide range of experimental animals (dog, pig, cow, horse, sheep, rat, rabbit) have been examined and no effect of porcine or bovine AHF has been shown on their platelets. The only animal platelets which give the same reaction to AHF as the human is the guinea pig. This confirms the observations of Sinakos and Caen (1967) who used a crude bovine fibrinogen preparation to produce platelet aggregation.

Despite wide usage of these preparations there has been no clinical evidence of pulmonary or myocardial complications

in patients receiving bovine or porcine AHF. In one case (Wardle, 1967), proteinuria developed during a course of bovine AHF which also produced thrombocytopenia. Proteinuria and biochemical evidence of renal damage was present in this patient three months later. In a case described by Goudemand et al. (1968) the patient developed a reversible episode of glomerulonephritis. The cause of this was obscure but it was suggested by the authors that it represented an immune reaction.

It is certain that many haemophiliacs owe their lives to the use of animal antihæmophilic factor, however, as more potent concentrates of human material become available the use of animal products will diminish but they still retain great interest as platelet aggregating agents in which role they seem to have unique actions.

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8. 1968 - 1970: Research Fellow, American Heart Association (with Dr. Oscar Ratnoff, Case-Western Reserve University, Cleveland, Ohio, U.S.A.). Fulbright Fellow.

of blood group antibodies and the effects of elevation of the plasma fibrinogen are discussed.

Animal AHF preparations have been used for some years in the management of haemophilia. In Chapter 10 are the studies carried out both in vivo and in vitro into the mechanism of platelet aggregation by preparations of animal AHF. I present evidence that the aggregating agent is the AHF molecule itself and not fibrinogen as had been thought previously. This work includes investigation of the physical and chemical characteristics of the aggregating agent as well as studies of inhibitors of the reaction.

products. From this study it is clear that patients with haemophilia may safely have teeth extracted after small doses of plasma concentrate and tranexamic acid.

Patients with haemophilia and other bleeding disorders are well known to have behaviour disturbances and on occasion florid psychiatric symptoms. One reflection of stress may be the high incidence of peptic ulceration. This often presents as gastro-intestinal bleeding and was one of the commonest causes of death. Chapter 6 is a review of the episodes of gastro-intestinal bleeding seen in this unit in the past ten years and covers one hundred and seven separate episodes of bleeding. In this I have confirmed that peptic ulcer occurs in over 50 per cent of these patients and may be symptomless. The hazards of conservative management and the complications of surgery are described.

The keystone of the modern treatment of haemophilia is replacement of the deficient activity by infusion of plasma or plasma concentrate. This exposes the haemophiliac to various additional hazards, of which serum hepatitis is the most important. In this section is described the incidence of both clinical jaundice and anicteric hepatitis, as well as the incidence of positivity of Australia (AuSH) antigen and antibody. In addition the incidence

therapy with plasma products the clinical signs in the acute haemarthrosis resolve rapidly but the radioactive scan remains abnormal. This suggests that the mechanism for production of pain (perhaps kinin generation) is different from that producing continuation of hyperaemia in the synovium.

Haematomata of muscle are usually situated peripherally and present no clinical diagnostic problem. Chapter 4 is concerned with attempts to diagnose early occult muscle bleeding by estimation of serum enzymes which are liberated from damaged muscle. The use of ultrasonography in the localisation and quantitation of peripheral haematomata is also investigated and new methods of investigation of chronic haematomata (pseudo-tumours) are described.

The commonest elective surgical procedure in the haemophilia is dental extraction. In recent years the availability of potent concentrates of plasma products have overcome the problems of excess bleeding from the extraction site but have introduced the hazards of production of serum hepatitis or AHF inhibitors. Chapter 5 is the report of a carefully controlled, double-blind trial of tranexamic acid, a fibrinolytic inhibitor, in the reduction of post-extraction bleeding and of the requirements of plasma

Perhaps the greatest cause of morbidity in haemophilia is recurrent bleeding into the joints of the severely affected patients. From studies both in the human and in the animal it would seem that a vicious circle is set up in which bleeding produces hypertrophy of the synovial villi and produces synovial hyperaemia; this in turn increases the chances of further bleeding and eventually bone and cartilage changes occur. It seems likely that if therapy is to be successful then it must be given early before cartilage or bone changes have occurred. It is known that plasma infusion will produce dramatic relief of the symptoms of acute haemarthrosis but as yet there is no evidence that it significantly alters the pathological processes in the joints. I have attempted to use the new techniques of radioactive joint scanning with technetium, and thermography in an attempt to define the early changes in the knee joints of haemophilic patients with acute and chronic joint disease and also with no apparent joint disease. These two techniques probably measure slightly different aspects of joint inflammation but both are more sensitive than clinical examination and detect changes before they are apparent radiologically. Both methods are easily repeatable and offer a method of assessing resolution of acute haemarthroses. Following

The Haemophilias: Clinical and Laboratory Investigations

Summary of M.D. Thesis submitted by Dr. Charles D. Forbes

The clinical features of haemorrhagic disease have been recognised for almost 1,500 years but it is only in the last fifty years that significant advances have been made in their diagnosis and treatment.

The advances of most importance concern the understanding of the normal coagulation and haemostatic mechanisms. It has become apparent in recent years that the "Bleeding Syndromes" consist of a large number of different diseases each with its own specific protein abnormality. In this thesis I look at the haemorrhagic problems of patients with haemophilia and Christmas disease. In these two diseases the clinical episodes of bleeding are identical although they are due to deficient activities of different proteins.

The thesis consists of three sections. In the first is the detailed historical introduction to the "Haemophilias" and the current concepts of the clotting mechanism. In the second section there are five chapters dealing with different clinical facets of bleeding in these patients and in the third section is described the complications and side effects of therapy with plasma products of human and animal origin.

THE HAEMOPHILIAS : CLINICAL AND LABORATORY
INVESTIGATIONS

by

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Thesis submitted for the degree of
Doctor of Medicine, University of Glasgow

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APPENDIX 4 ADDITIONAL CASE HISTORY
(A4.1 - A4.7)

APPENDIX 1

FIGURES

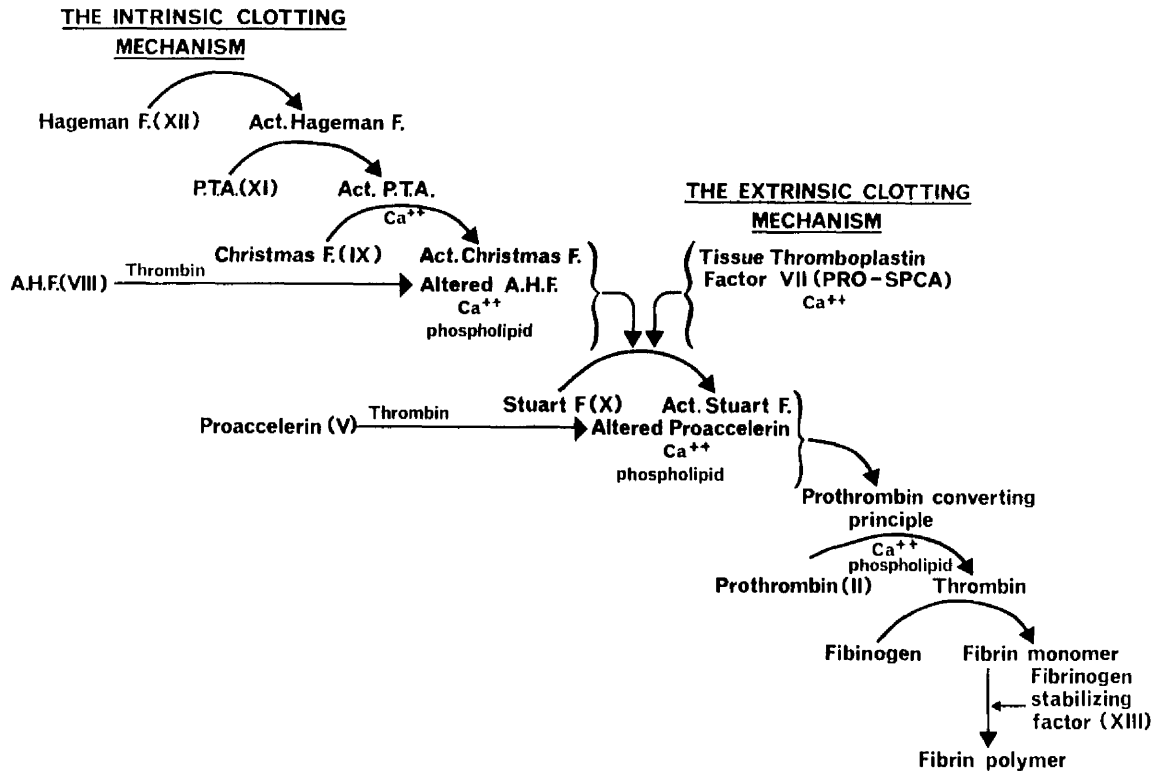


Fig. 1 Diagrammatic representation of the "waterfall" or "cascade" theory of blood coagulation as put forward originally by Macfarlane (1964) and Davie and Ratnoff (1964). The original sequence of events has been modified slightly in the light of modern knowledge.

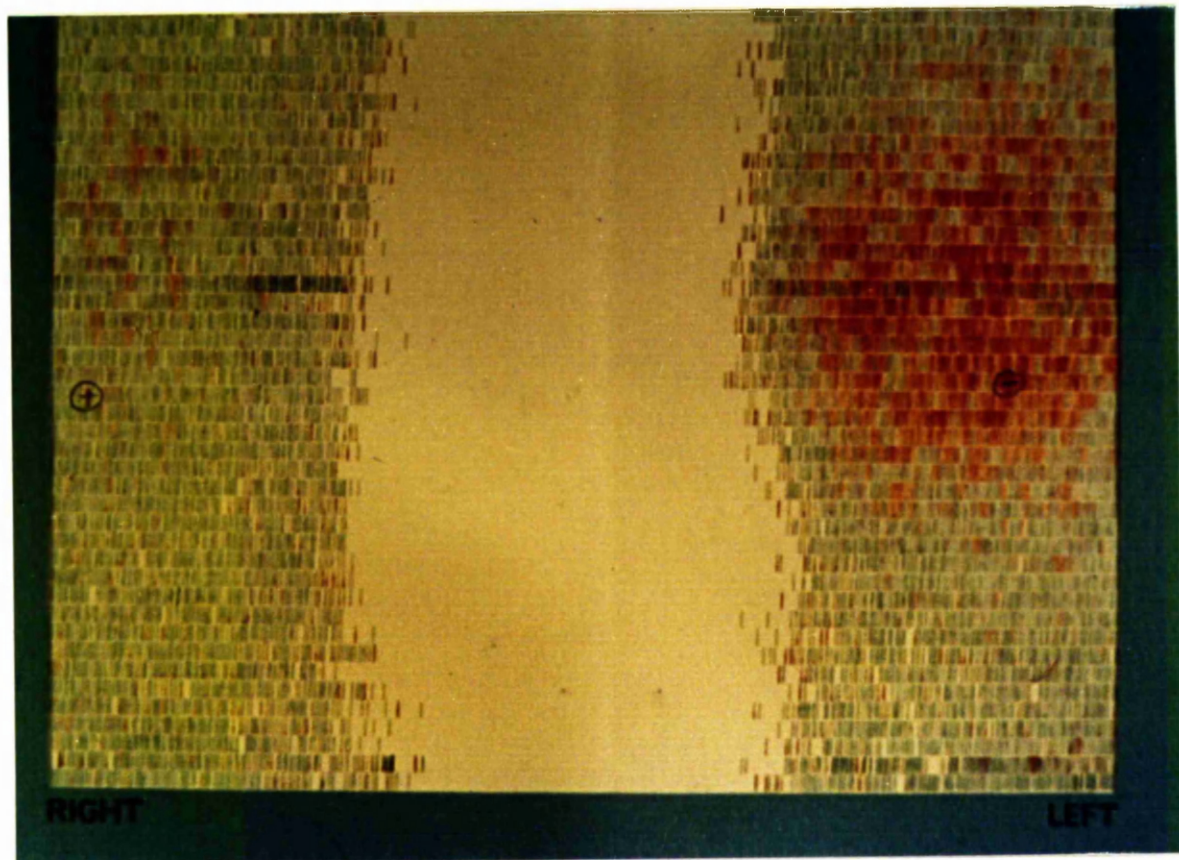


Fig. 2 Representative colour scan of haemophilic knee joints obtained twenty-five minutes after the intravenous injection of 5 mc. of ^{99m}Tc . Colour coding is so arranged that there is a gradation from deep red, red, orange, yellow, purple, blue, green, brown which corresponds to diminishing amounts of radio-activity. There is no isotope uptake in the right knee which has never been the site of a haemarthrosis and was clinically and radiologically normal. It has been given a score of 0. The left knee has a score of 2.

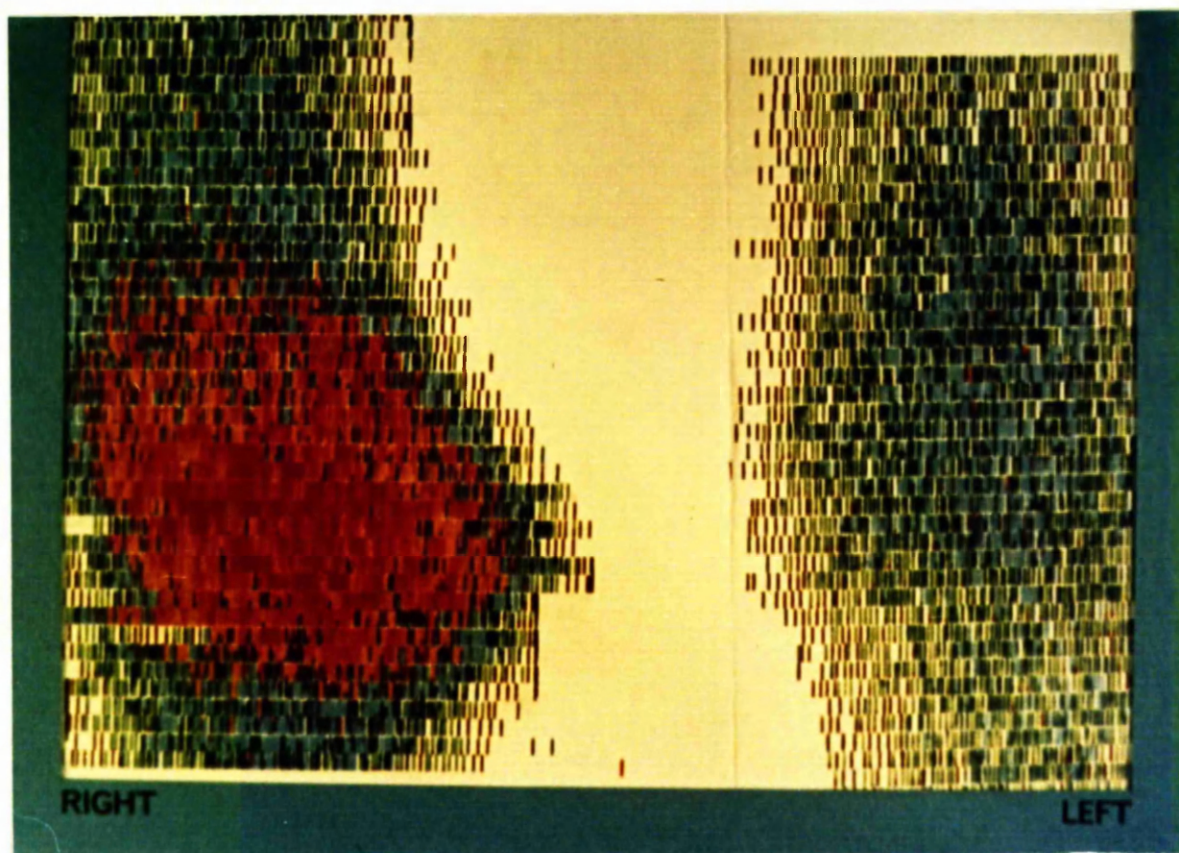


Fig. 3 Representative colour scan of a patient with severe haemophilia with an acute haemarthrosis in the right knee joint (patient 1). This scan has been given a score of 3 using our scoring technique which depends on the width, depth, degree or isotope uptake with or without irregular localisation of the isotope. The left knee has a score of 0.

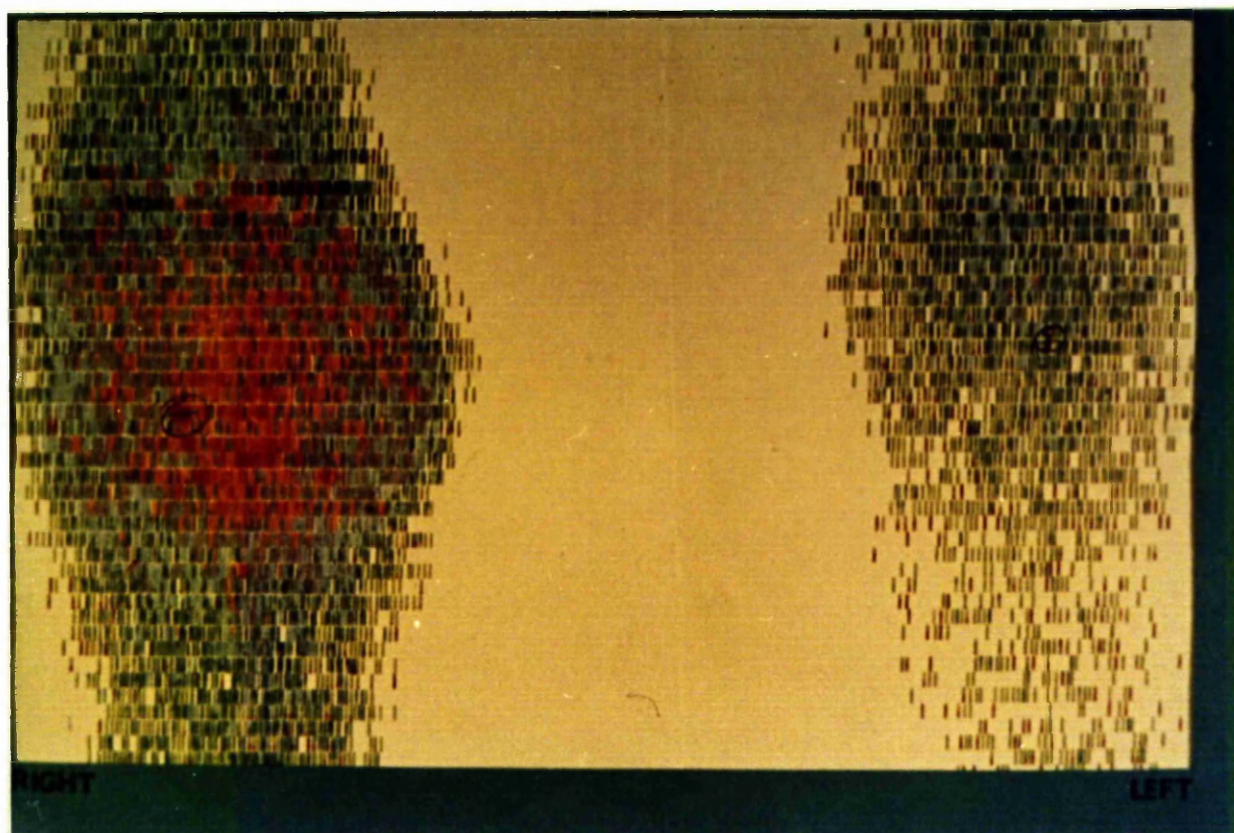


Fig. 4 Representative colour scan of a patient with severe haemophilia and chronic knee joint disease (patient 10). The scan of the right knee has been graded 2 on the basis of the isotope uptake. The left knee has a score of 0.

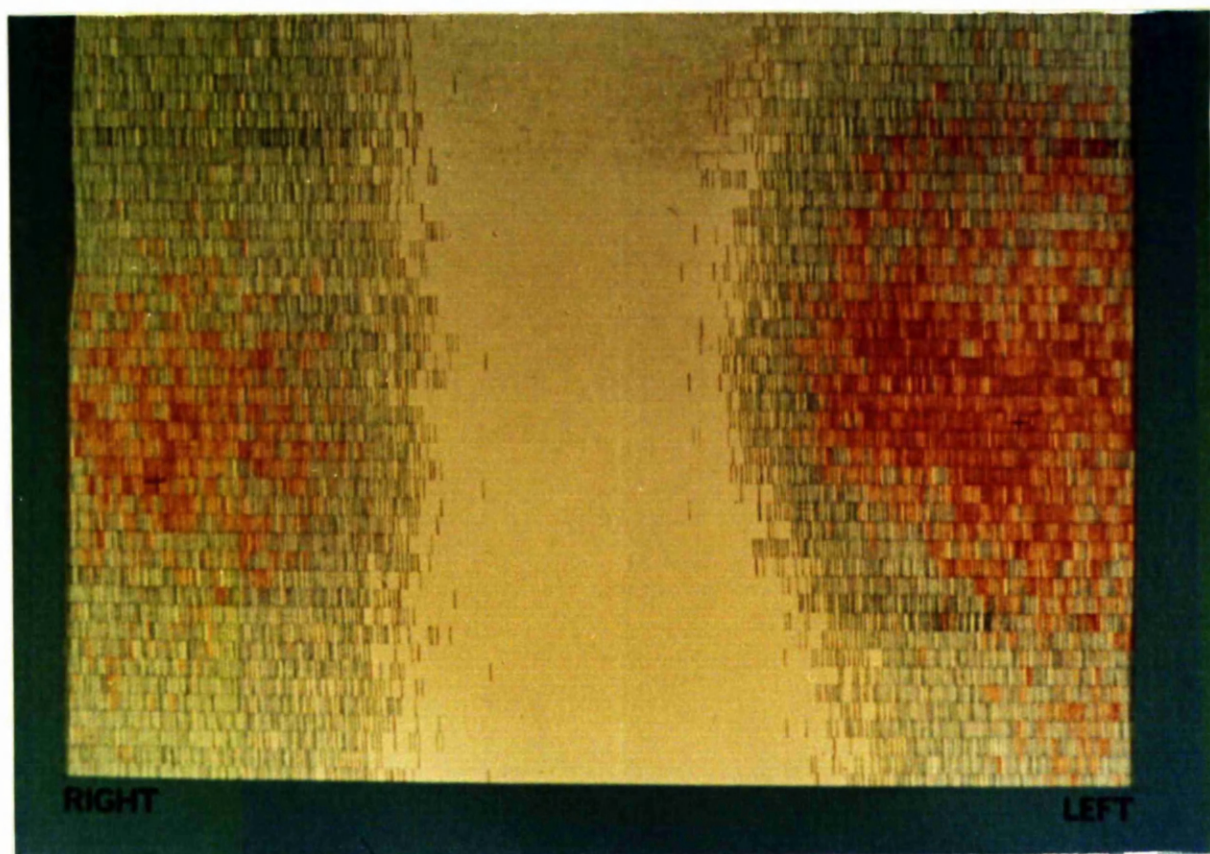


Fig. 5 Representative colour scan of a patient with severe haemophilia showing bilateral abnormalities in the knee joints (patient 5). The right knee joint has been given a score of 1 and the left knee a score of 3.

Acute joint disease - 6 patients

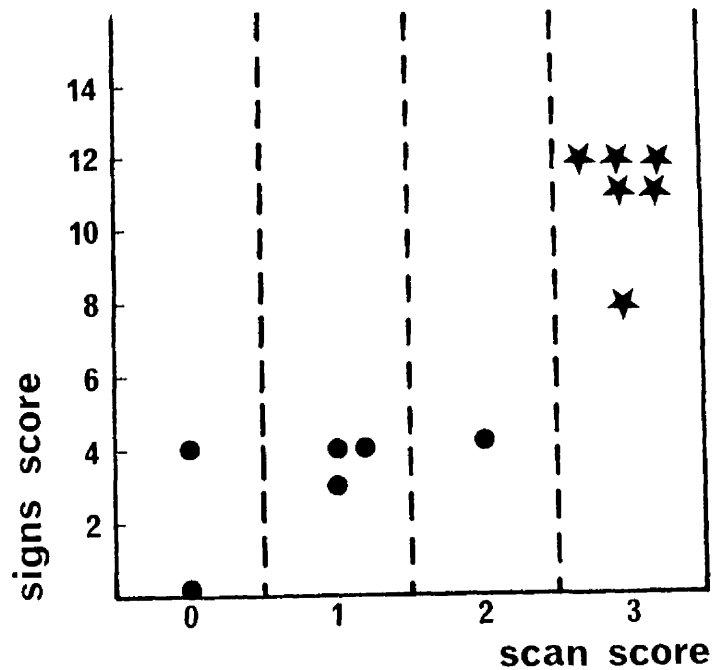


Fig. 6 Relation between the clinical 'signs' score and the radio-active scan abnormalities in acute haemophilic haemarthrosis. There is a good correlation between the scan abnormality and the 'signs' score. For comparison the other joint of the patient is shown. All these joints were the site of frequent previous bleeding episodes and all had evidence, both clinical and radiological, of chronic haemophilic arthropathy.

☆ - acute haemarthrosis ● - chronic joint disease.

Chronic joint disease -15 patients

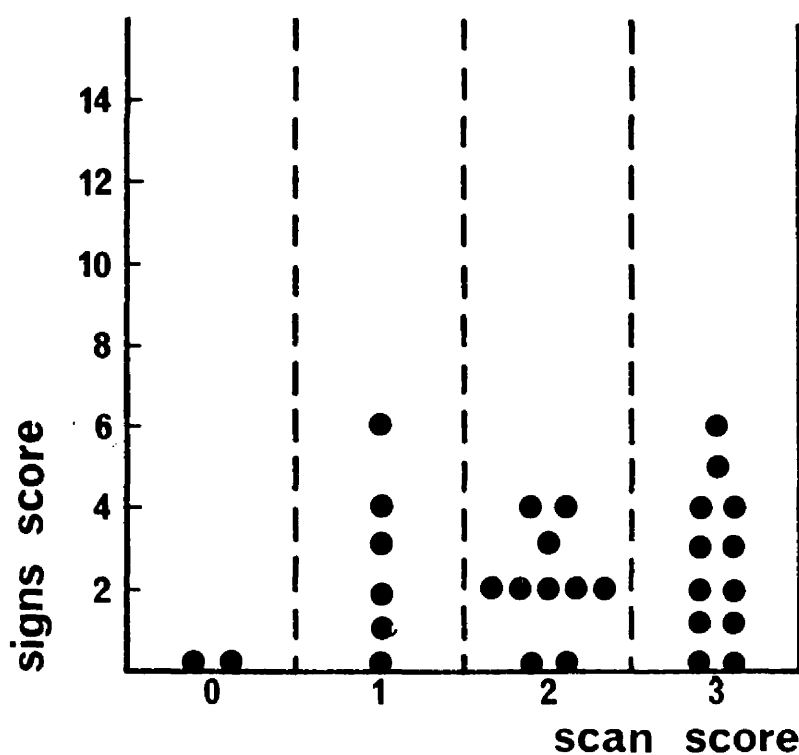


Fig. 7 Relation between the clinical 'signs' score and the radioactive scan in chronic haemophilic joint disease. There is a poor correlation between the clinical 'signs' score and the abnormality of the scan.

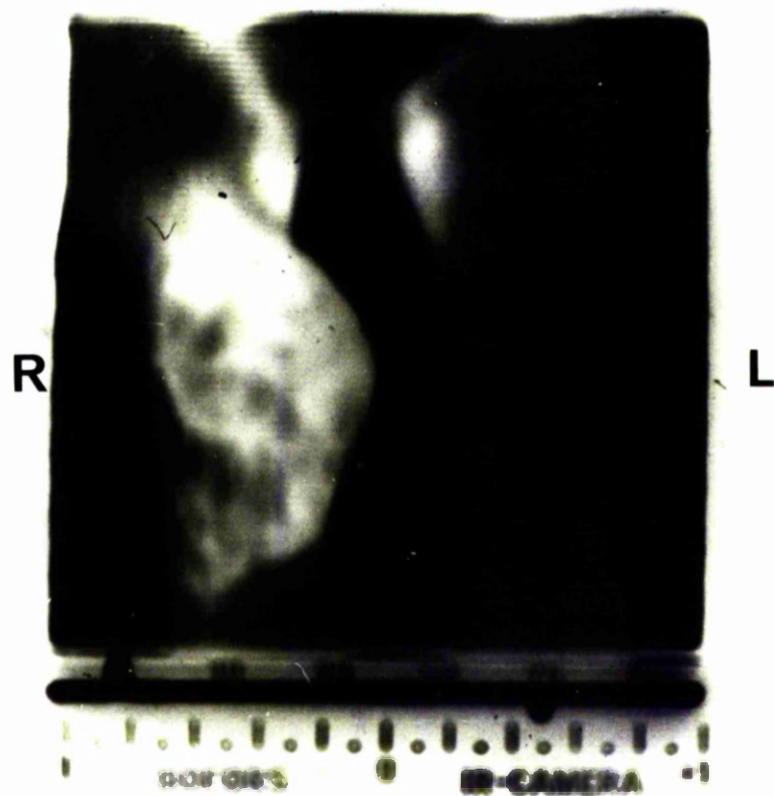


Fig. 8 Thermograph of the knee joints of a patient with severe haemophilia with an acute haemarthrosis in the right knee joint. This has been given a score of 3. There is irregular emission of infra-red energy from this joint and the swelling of the joint and wasting of the quadriceps can be seen. The left knee is normal.

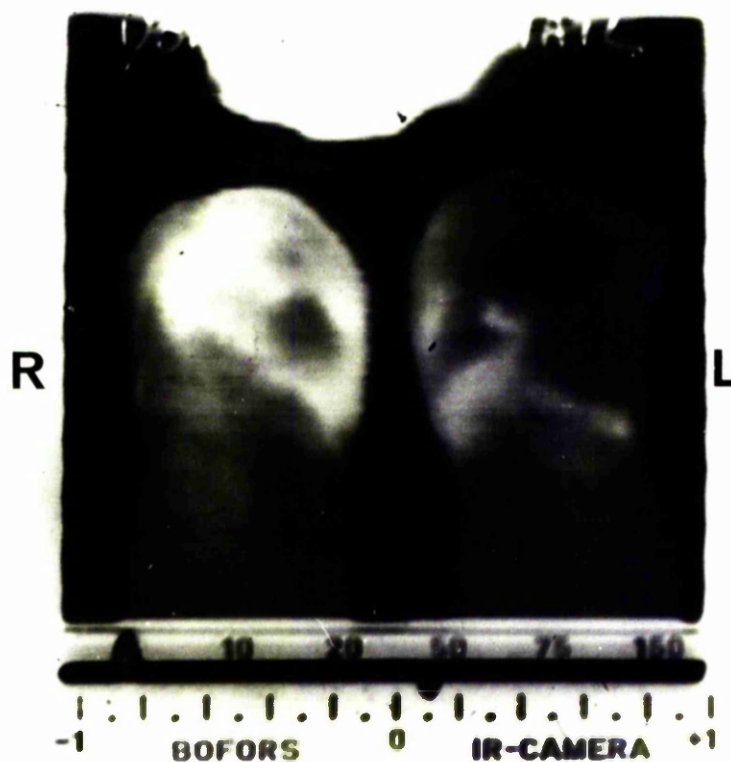


Fig. 9 Thermograph of the knees of a patient (patient 9) with severe haemophilia and chronic arthropathy of the knees. Both joints are abnormal, the right knee has been given a score of 2 and the left a score of 1. There is irregular emission of infra-red energy from both knees. This may be due, in part, to abnormal venous plexuses around the disorganised joint.

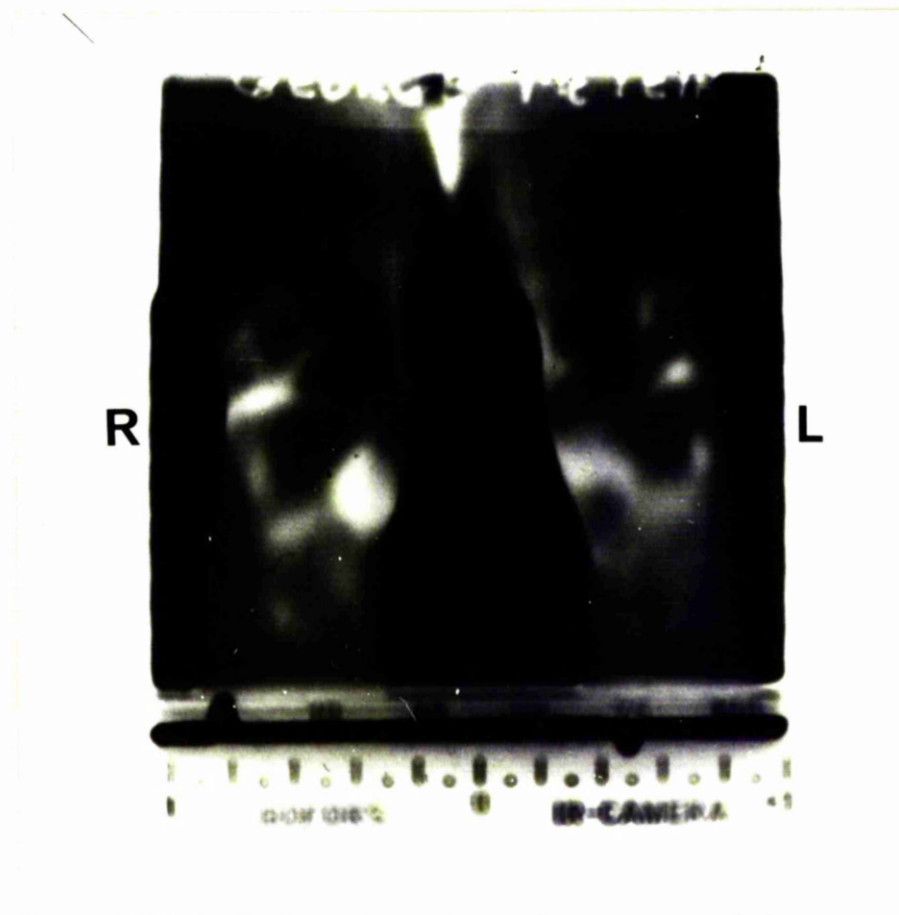


Fig. 10 Thermograph of the knees from a patient (patient 13) with mild haemophilia who has never bled into the knee joints and who has no clinical abnormalities. There is abnormal infra-red emission from both knees and this has been given a score of 1.



Fig. 11 Thermograph of the knees from a patient (patient 1) with severe haemophilia who has an acute haemarthrosis of the right knee. The left knee appears normal and has been given a score of 0. The right knee has a score of 3. The use of isotherm lines to determine the difference in temperature between these two joints is shown in the next figure.

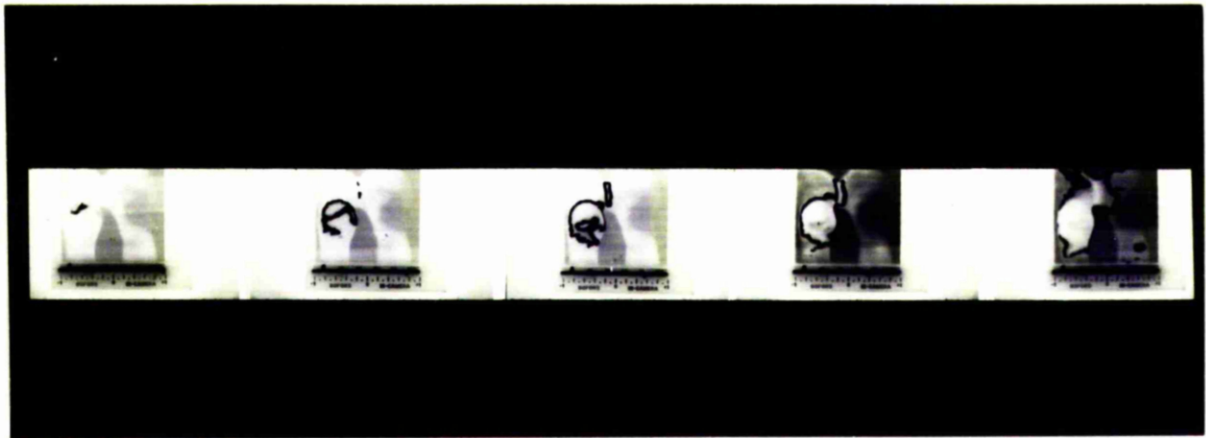


Fig. 12 Demonstration of the use of iso-therms to determine the difference in temperature between knee joints. As can be seen there is an acute haemarthrosis of the right knee joint; each of these pictures represents a step of 1°C and there is a 5°C difference between the two joints.

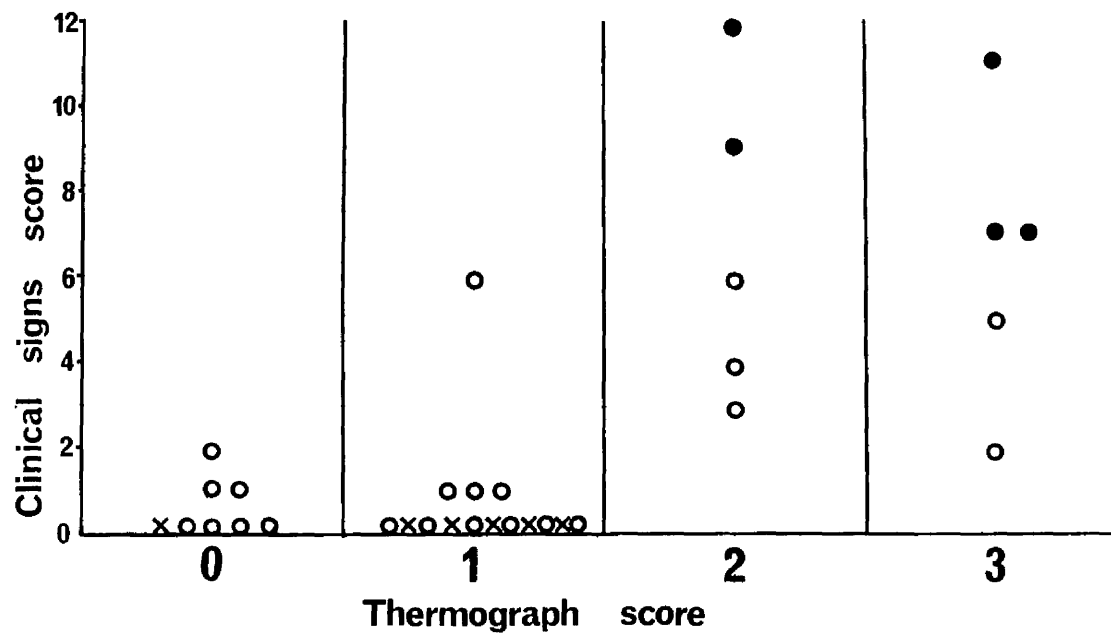


Fig. 13 Relation between the clinical 'signs' scores and thermography scores in acute and chronic haemophilic arthropathy and in patients with no joint disease.

- - represents acute haemarthrosis
- o - represents chronic joint disease
- x - no apparent joint disease

Acute joint disease - 5 patients

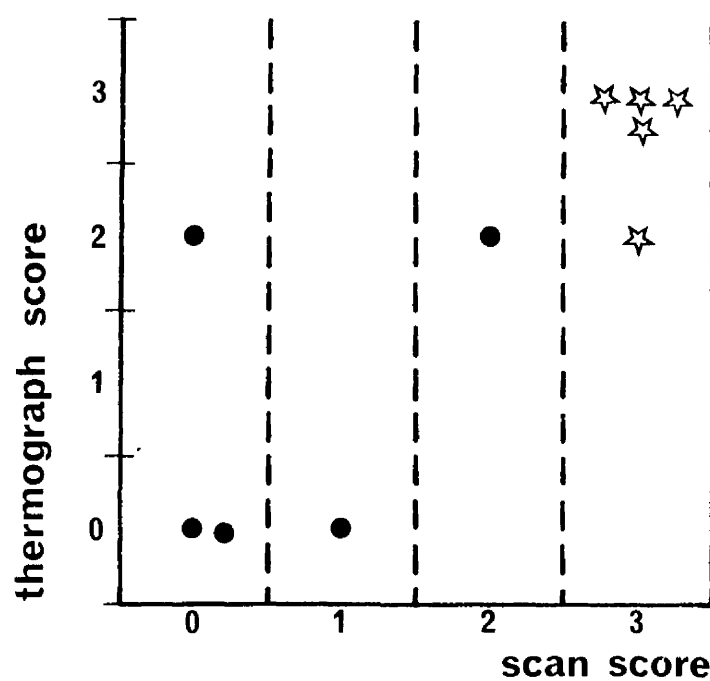


Fig. 14 Comparison of thermographs and radioactive scans in acute haemarthrosis. The opposite joint, which has chronic disease, is shown for comparison. There is a good correlation between the scans and thermographs in the acute joints.

- ☆ - acute haemarthrosis
- - chronic joint disease

Chronic joint disease - 6 patients

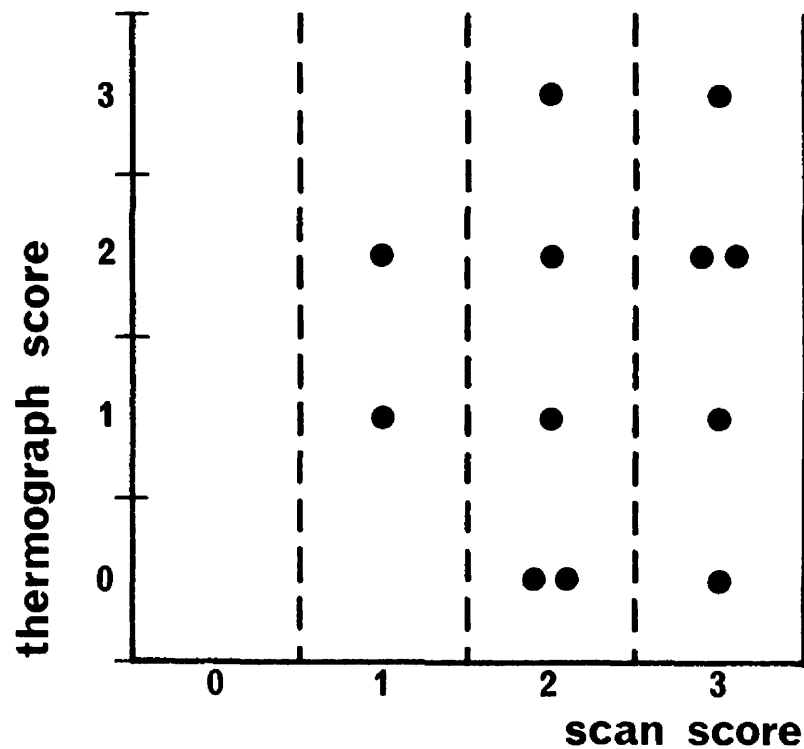


Fig. 15 Comparison of thermographs and radioactive scans in chronic haemophilic joint disease. There is a poor correlation between the scans and the thermographs. It is possible that the scan and thermograph measure different aspects of joint inflammation in this situation (see text).

Number of patients

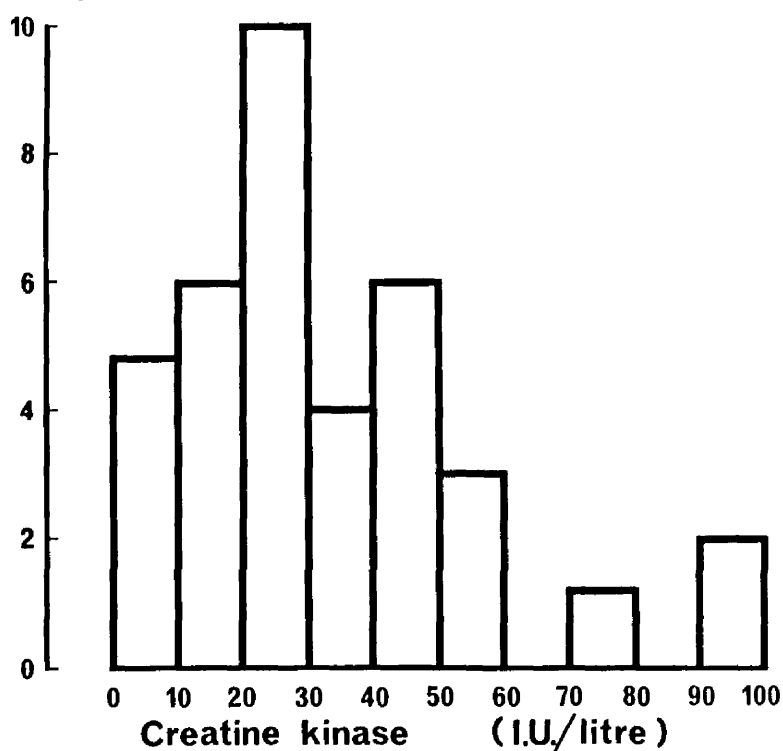


Fig. 16 Distribution of creatine kinase activity in forty-one patients with haemophilia or Christmas disease who had no evidence of muscle disease or muscle bleeding. There is no significant difference between this group of patients and the normal levels reported by MacQueen et al. (1972).

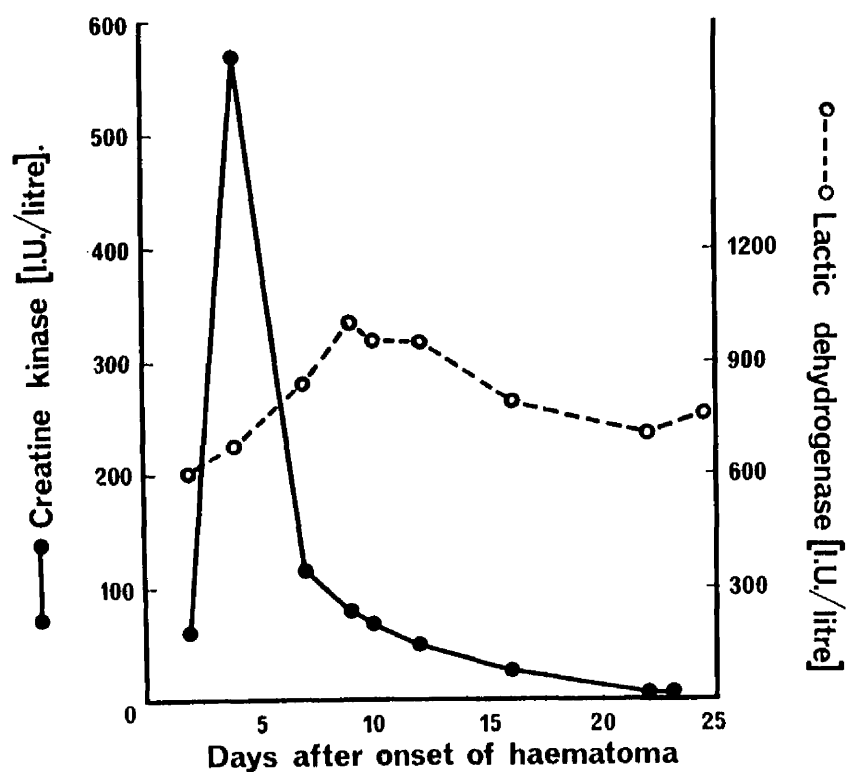


Fig. 17 Representative figure to show the changes in creatine kinase and lactate dehydrogenase after muscle bleeding in a patient who developed a massive muscle haematoma after an intramuscular injection (patient 2). The peak activity of CK in this patient occurred at 96 hours. Peak LDH activity occurred on day 10. Electrophoresis and examination of isoenzymes showed a pattern 1 > 2 > 3 > 5 > 4 suggesting that the increase in LDH resulted from breakdown of red blood cells within the haematoma.

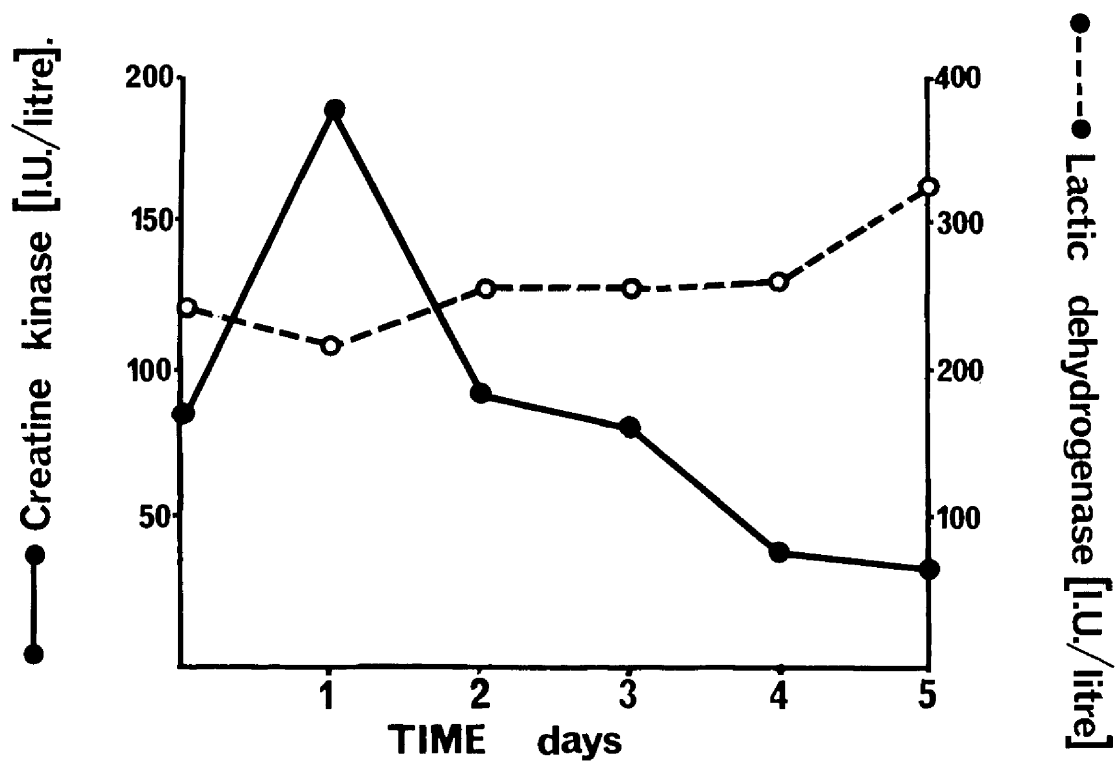


Fig. 18 Pattern of elevation of CK and LDH in a patient with severe Christmas disease who presented with loin pain of undetermined aetiology.

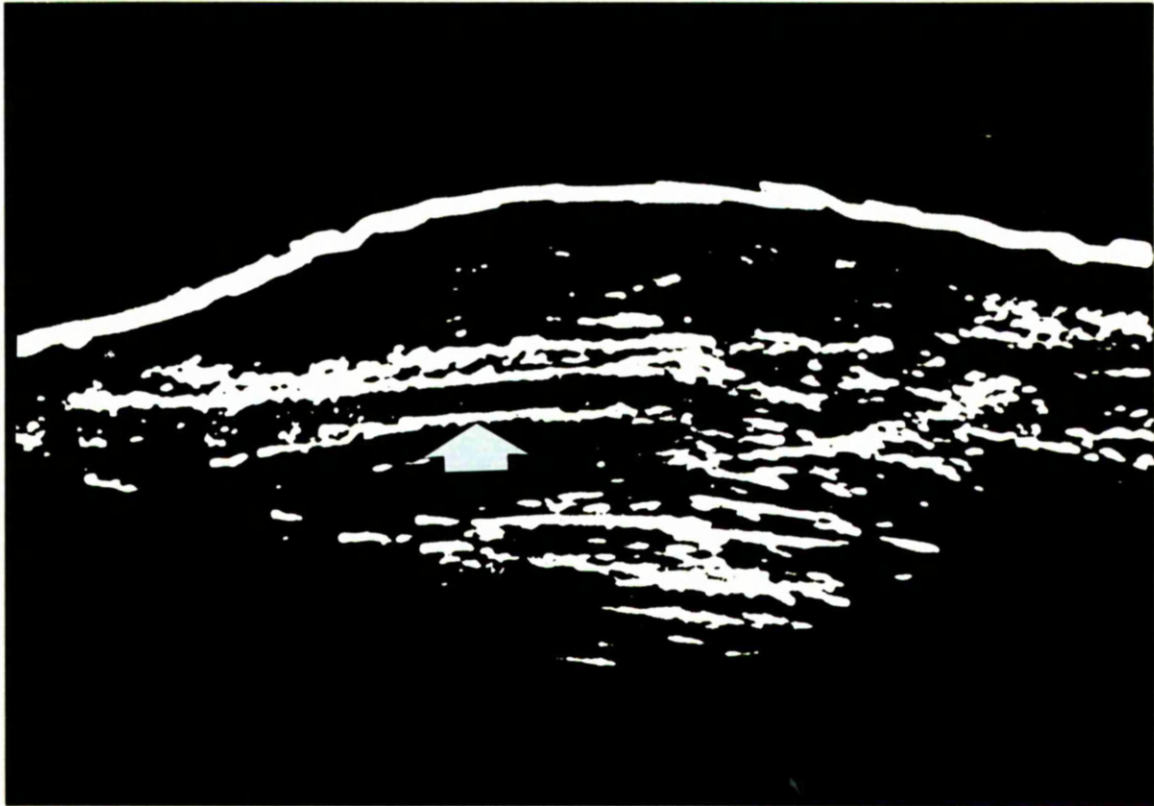


Fig. 19 (a) Ultrasonograph (1.5 MHz, - 20) of thigh in patient 2. Longitudinal section of lateral aspect of thigh showing the extent of the haematoma (35 x 6 cms). The position of the femur is shown by the arrow. There are reflecting echoes within the haematoma.

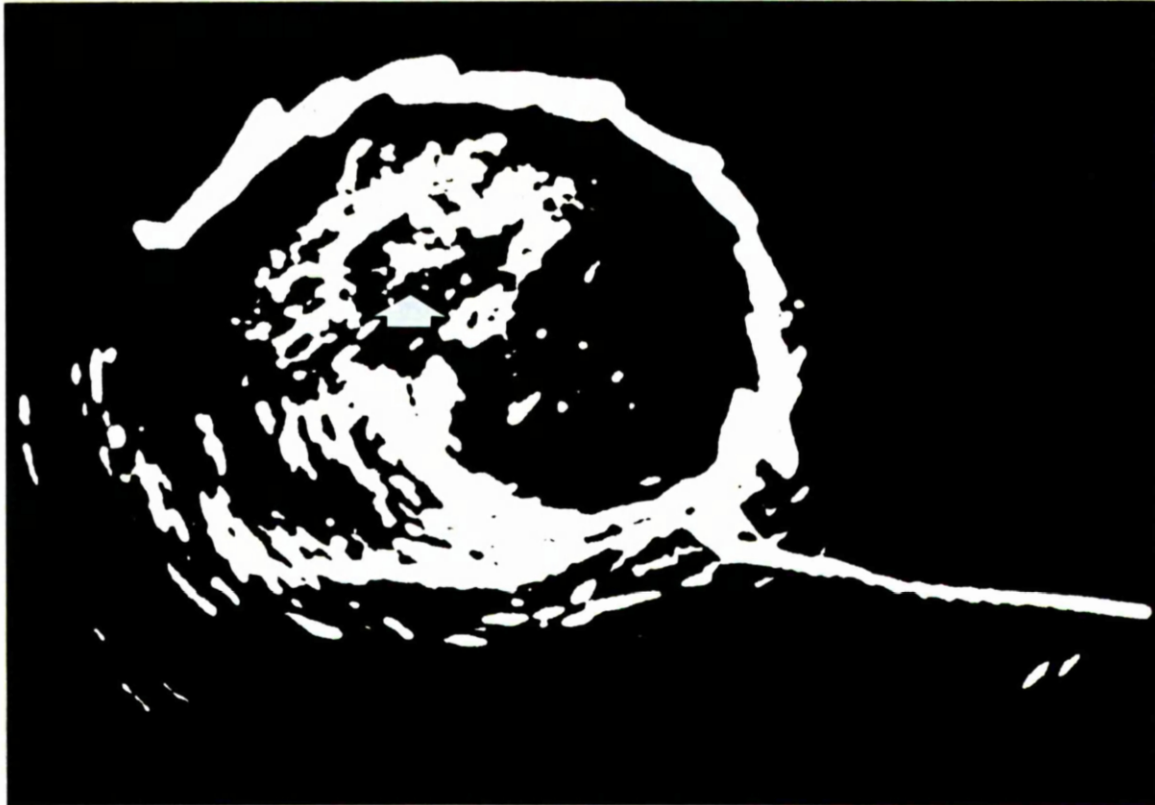


Fig. 19 (b) Ultrasonograph (1.5 MHz, - 20) of thigh in patient 2. Cross section of thigh 6.5 inches above the patella to show the large transdonic space filled by the haematoma (15 x 20 cms). The position of the femur is shown by the arrow.

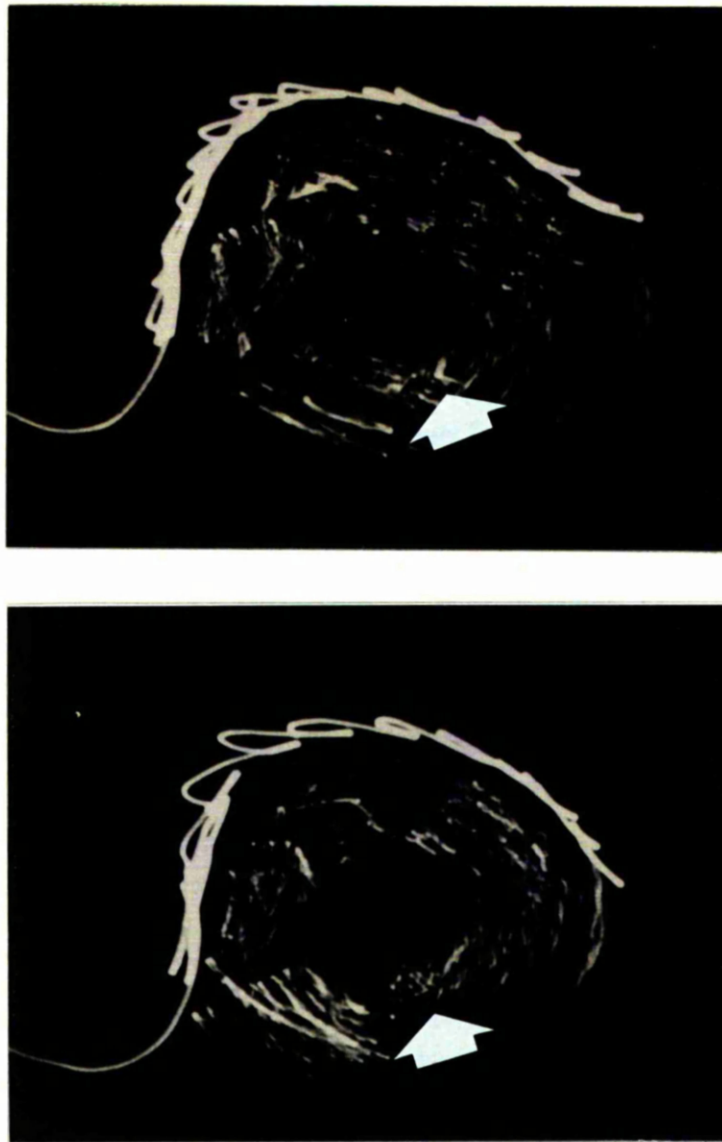


Fig. 20 (a) and (b) Ultrasonographs of the thigh in patient 5 showing the presence of a large haematoma (cross section 5 x 6 cms) in the posterior compartment of the thigh extending from the knee into the buttock. Fig. 20 (a) is taken at 16 cms above the patella and 20 (b) at 10 cms above the patella. Both figures show transverse sections of the thigh and were taken at 2.5 MHz, - 20.

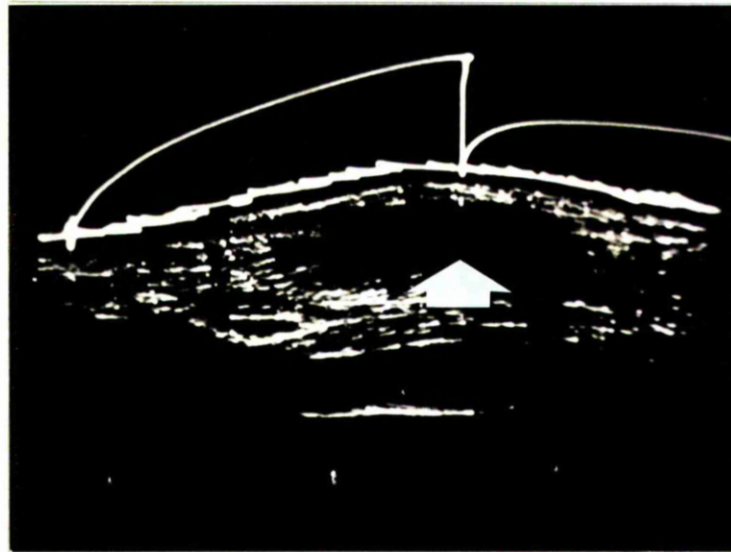


Fig. 21 Ultrasonograph of calf in patient 3. Scan taken with patient lying on face so that posterior surface of calf is uppermost. Central mark indicates the site of cutaneous bruising where the patient was kicked while playing football. The foot lies to the right of the picture. The cross section of the haematoma measures 15 x 5 cms. Scan taken at 2.5 MHz (-30). Arrow points to the haematoma.

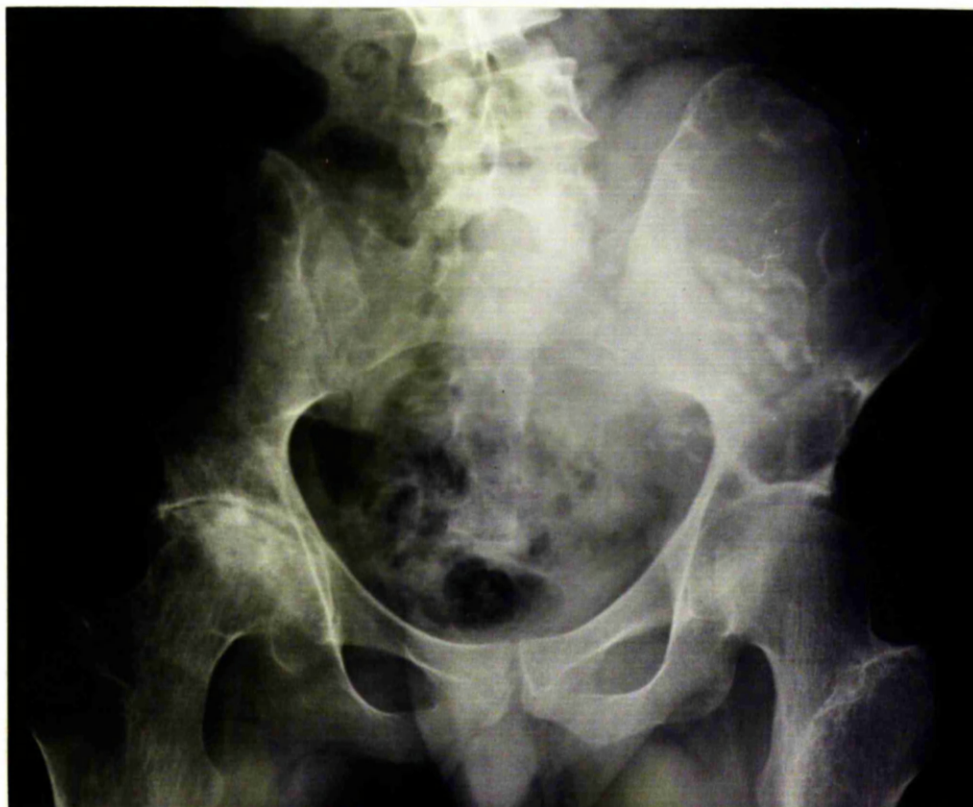


Fig 22 Plain X-ray of the pelvis in a patient with bilateral pseudo-tumours. There is erosion of the blade of the ilium on the left side. Continuous with this area of bone destruction there is a soft tissue mass within which are areas of calcification. There are osteosclerotic areas in the ilium.

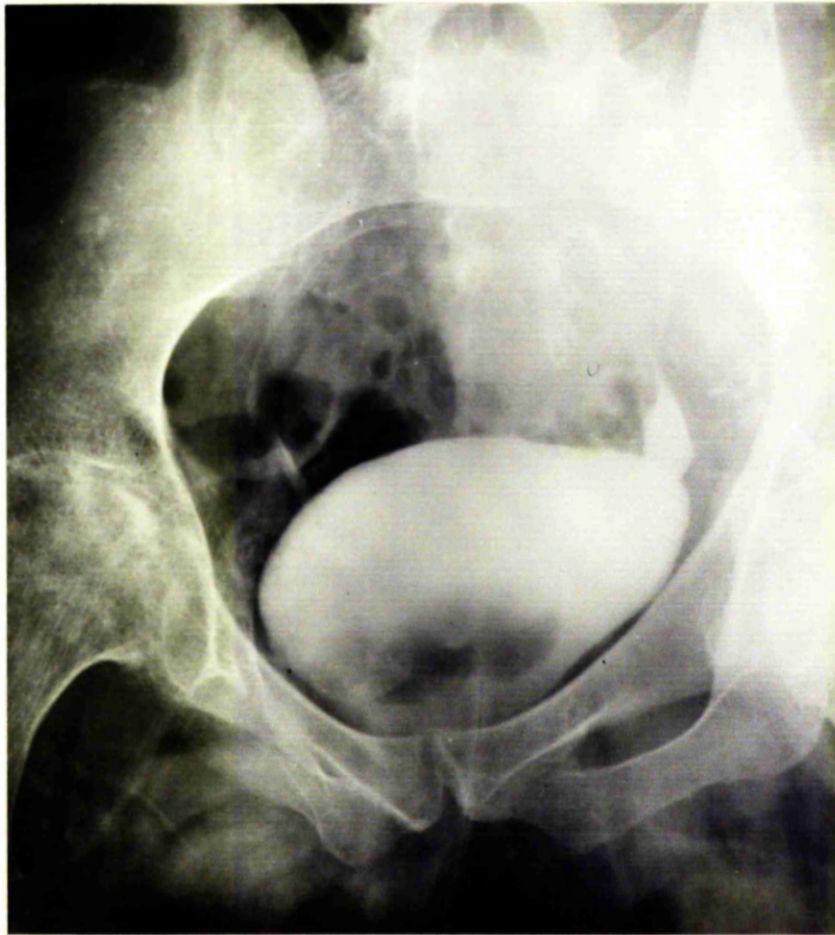


Fig. 23 (a) Intravenous pyelogram of patient with bilateral pelvic pseudotumours showing displacement of the left ureter and bladder. The left ureter is held in fixed dilatation by the pseudotumour.



Fig. 23 (b) Barium meal and follow through showing displacement of both large and small bowel by a large mass in the left pelvis and a smaller mass in the right side.



Fig. 24 Ultrasonograph of the lower abdomen (2.5 MHz, - 10) in the patient with bilateral pelvic pseudotumours. This is a transverse section of the abdomen 1 inch below the umbilicus showing two discrete lesions, measuring 7.5 x 5 cms on the right and 10 x 7.5 cms on the left. The margins of the lesions are indistinct and they contain reflecting echoes.

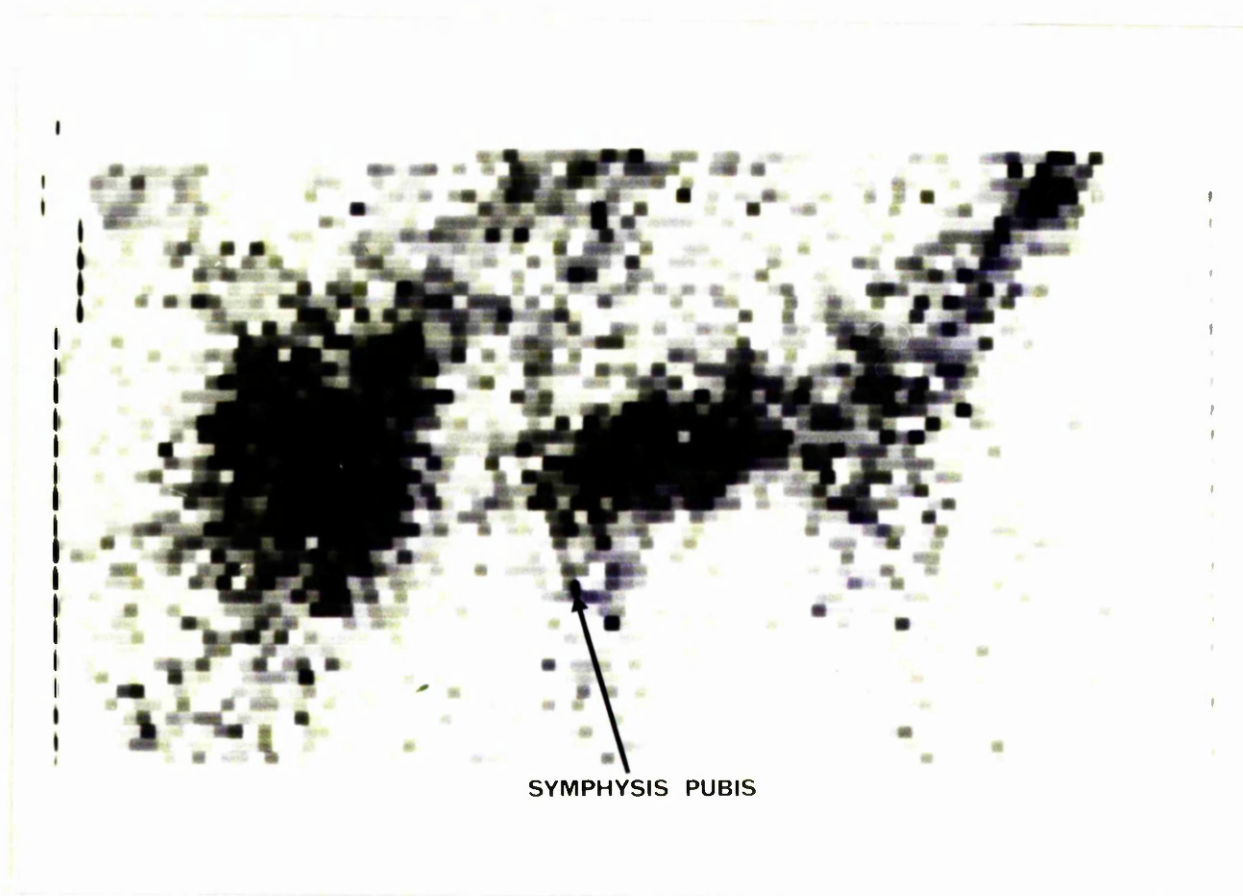


Fig. 25 Photostatic scan of pelvis after injection of ^{87}Sr showing a large round volume of abnormal uptake in the right pelvis and an elongated volume of abnormal uptake extending laterally and superiorly in the left pelvis. These areas correspond to the sides of the pseudotumour.

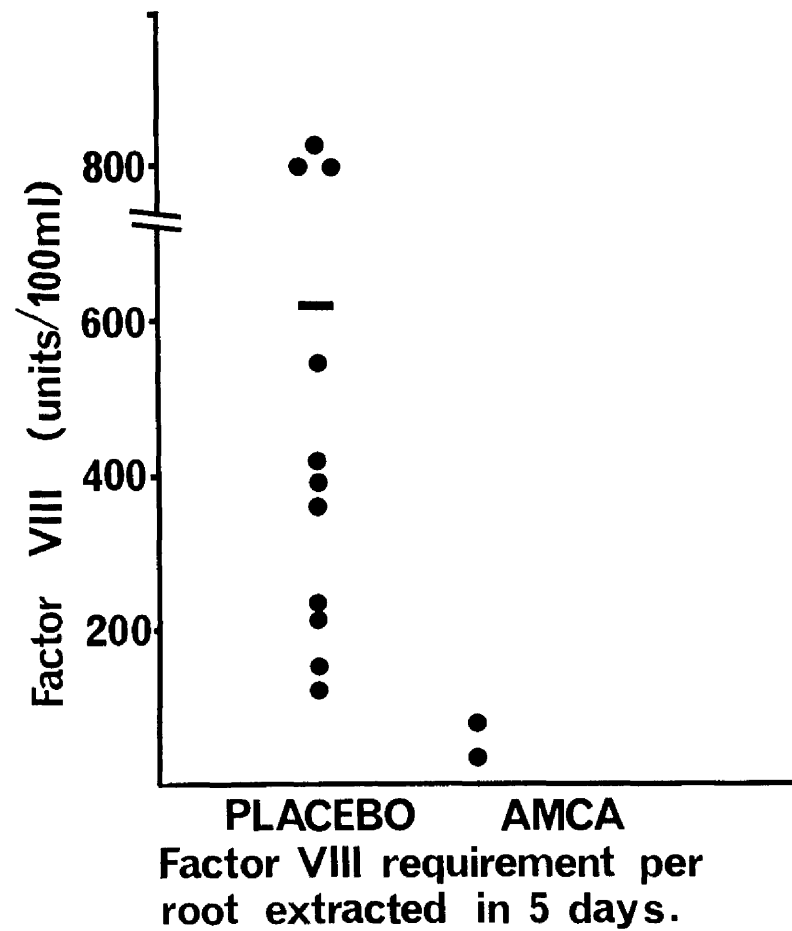


Fig. 26 Factor VIII replacement given after dental extraction to patients receiving tranexamic acid or placebo. In the treated group only two patients required to have additional replacement therapy and in the placebo group eleven required an average of 620 units of factor VIII.

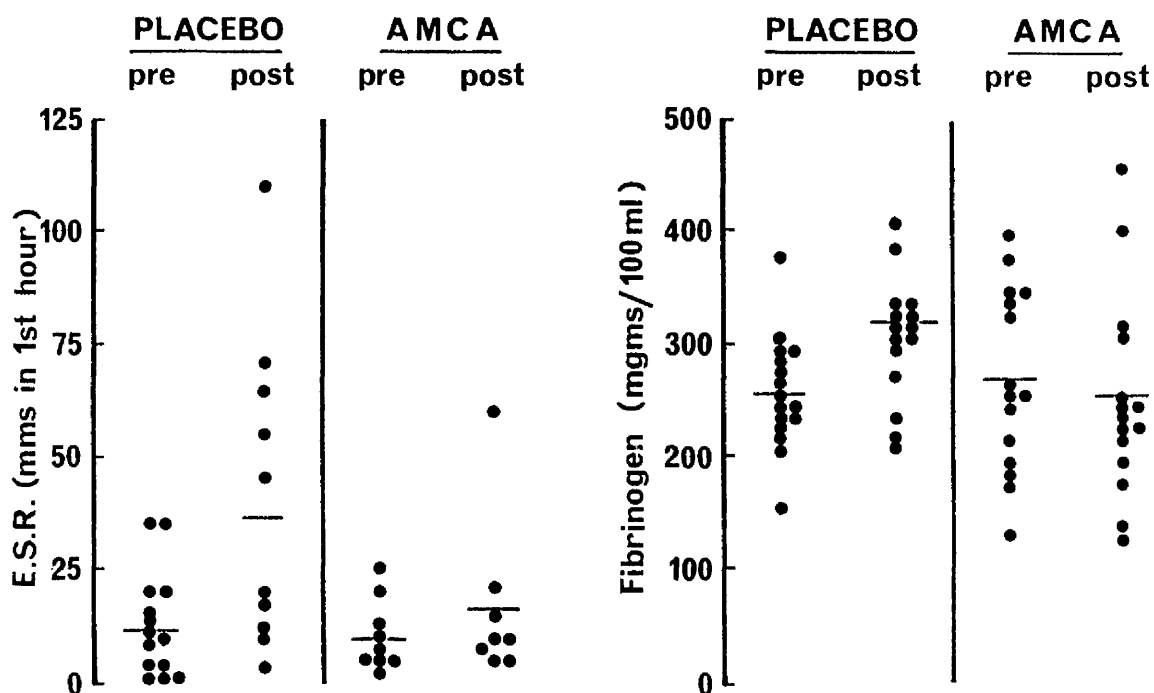


Fig. 27 Changes in the erythrocyte sedimentation rate and fibrinogen levels in the tranexamic acid treated and placebo groups. The significant rise in ESR and fibrinogen is a reflection of the volume of plasma concentrate infused.

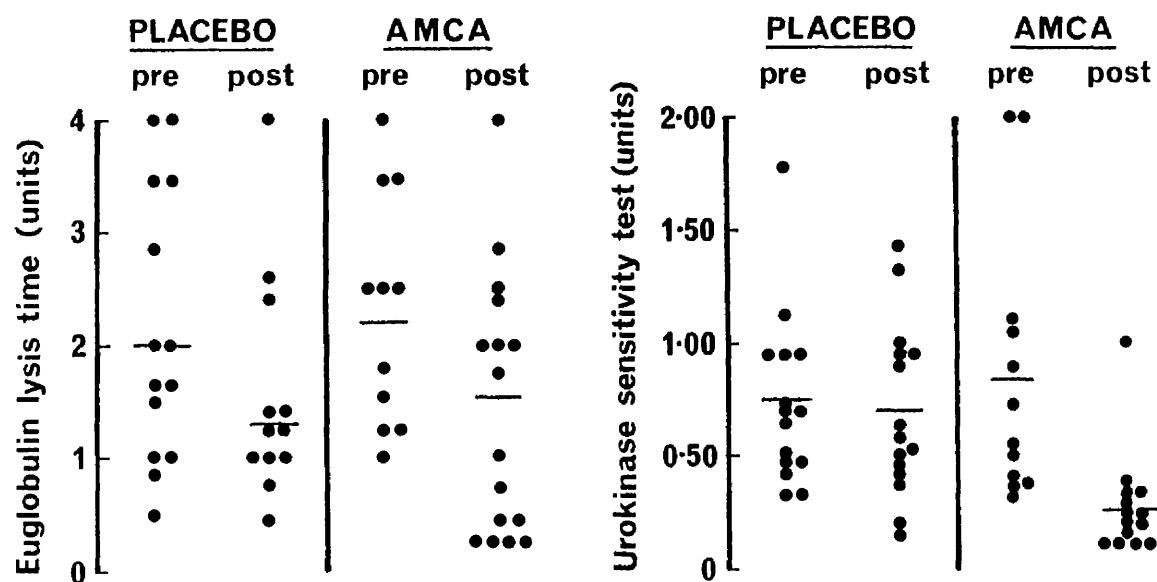


Fig 28 Urokinase sensitivity tests and euglobulin lysis time estimations before and after treatment with tranexamic acid and in the placebo group. Depression of urokinase sensitivity indicates that the plasma level of tranexamic acid was sufficient to inhibit fibrinolysis. In both groups there was a fall in the euglobulin lysis time indicating accelerated lysis but the differences were not significant ($P = 0.1$).

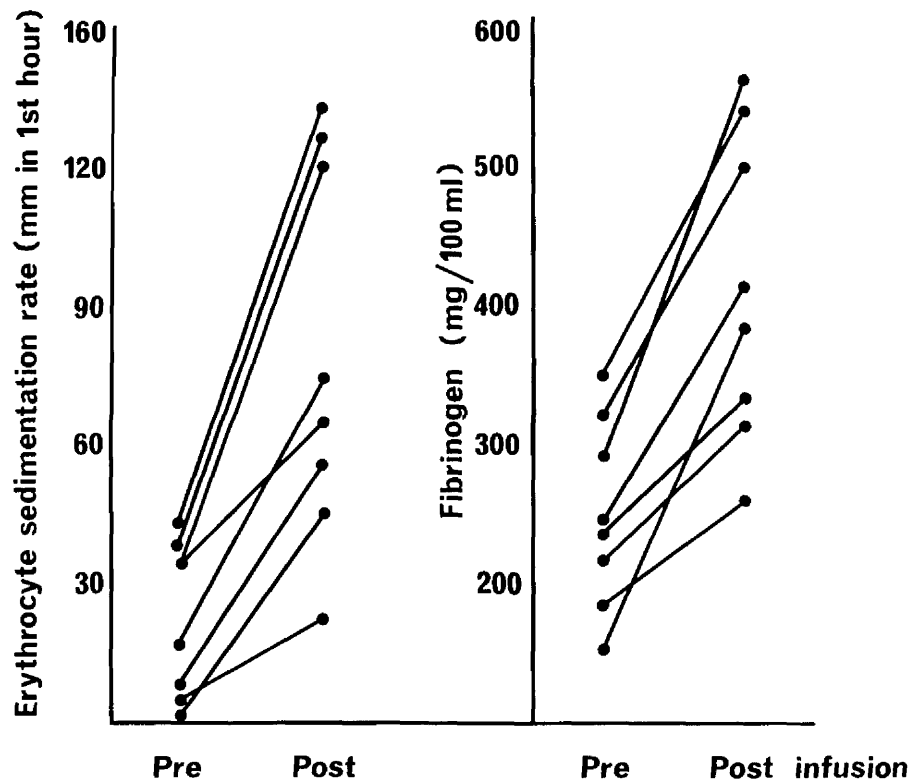


Fig. 29 Erythrocyte sedimentation rate and fibrinogen levels before and after cryoprecipitate infusion in eight patients. In both tests there are significant differences pre and post-infusion ($P = < 0.001$).

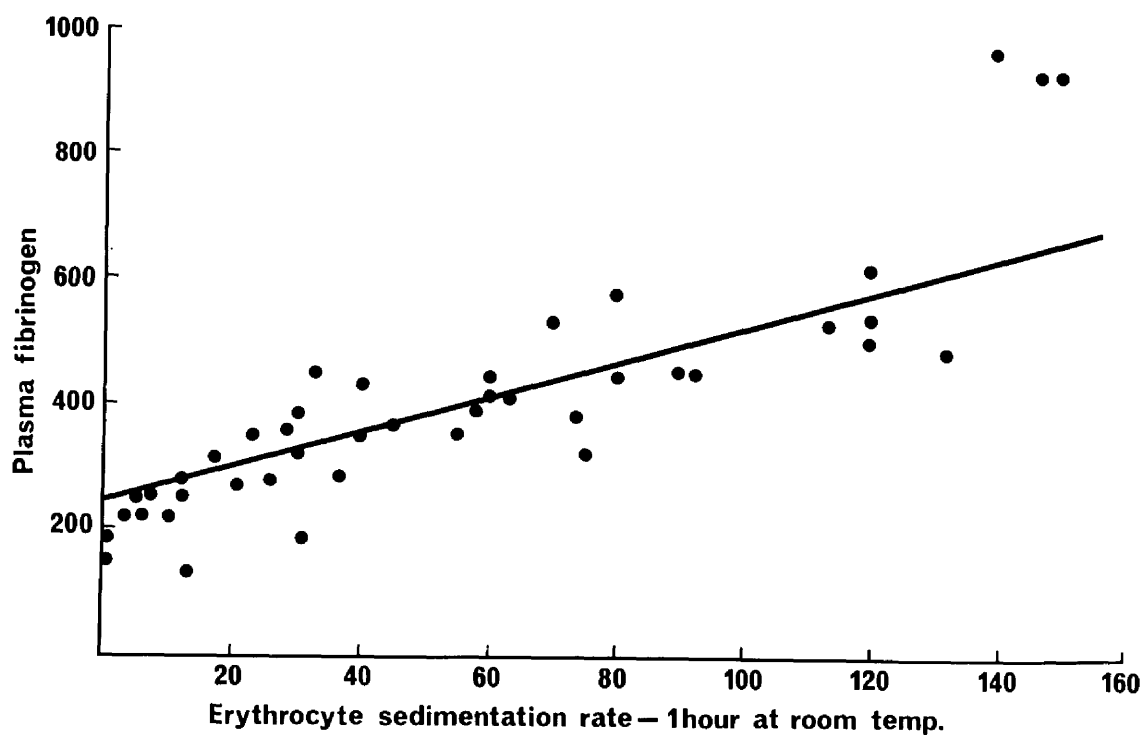


Fig. 30 Shows the relationship between fibrinogen and erythrocyte sedimentation rate ($r = 0.3255$). With a rise in plasma fibrinogen there is a corresponding rise in the ESR until it reaches a level which approximates to the packed cell volume, at which point no change in the ESR occurs with a further rise in fibrinogen level.

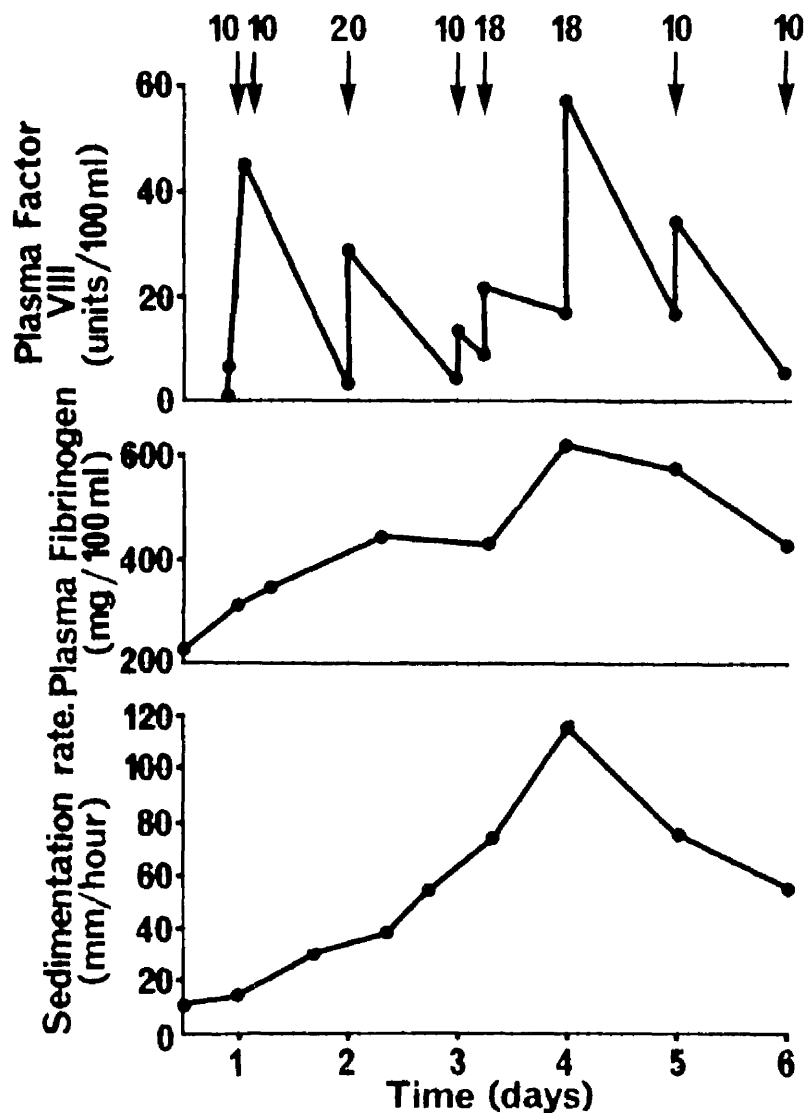


Fig. 31 Serial estimations of erythrocyte sedimentation rate, plasma fibrinogen and factor VIII levels in one representative patient who received multiple infusions of cryoprecipitate during surgery. The amount of cryoprecipitate given is indicated at the top of the slide. The peak level of fibrinogen and ESR occurred on day 4.

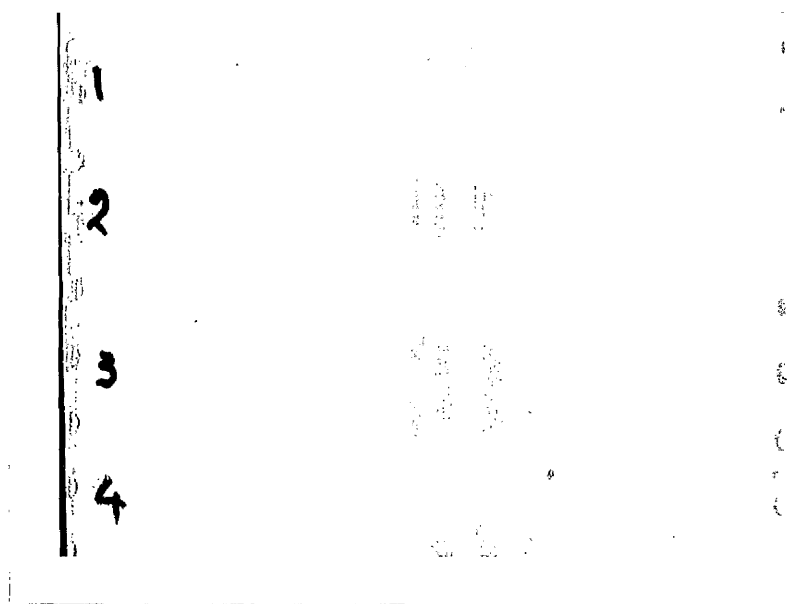


Fig. 32 Cellulose acetate electrophoresis of LDH iso-enzymes. 1, day 14; 2, day 15; 3, day 19; 4, day 20. Zones of activity were made visible by incubating the strips in contact with agar, tris-buffered to pH 8.4 and incorporating, per litre, 100 m. mol of lactate, 1.5 m. mol of NAD, 66 μ mol of phenazine methosulphate, 500 μ mol of thiazolyl tetrazolium and 6 μ mol of semicarbazide hydrochloride. This figure has been retouched to bring out the bands more clearly. The original photograph may be seen in our article in *Clinical Chemistry* (1971), 17, 948.

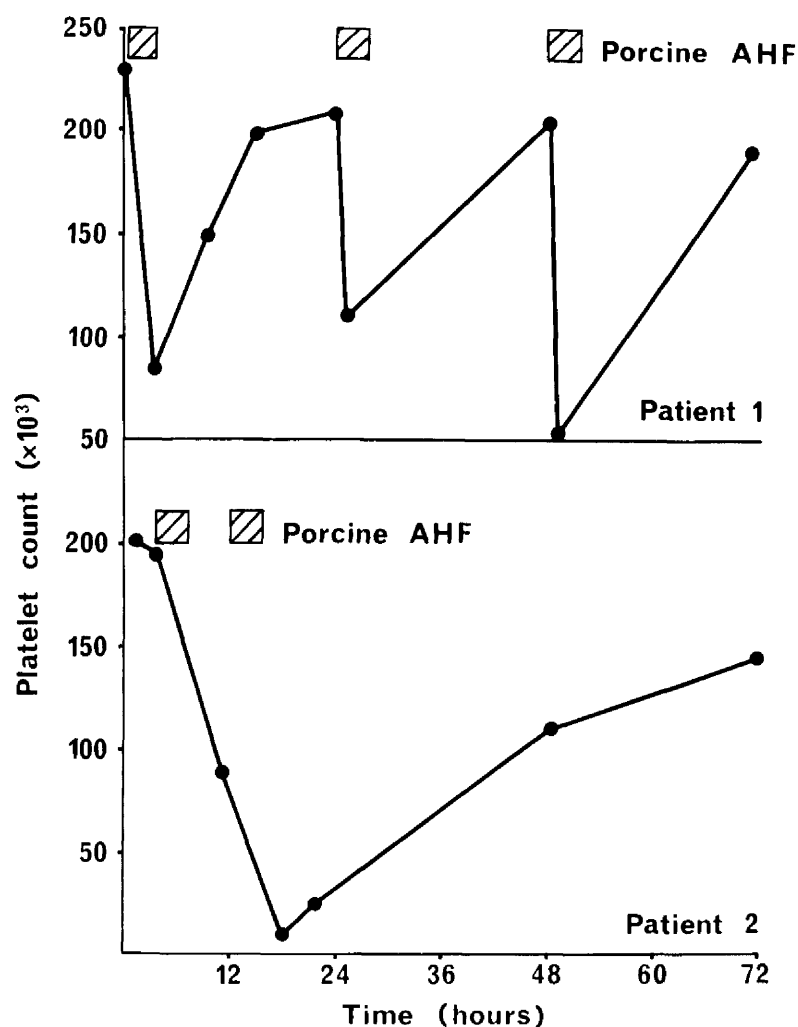


Fig. 33 Effect on the platelet counts of infusion of 8,000 unit doses (▨) of porcine AHF in two patients with haemophilia. Thrombocytopenia was noted 10 minutes after the injection and examination of peripheral blood films at this time showed the presence of circulating platelet clumps of 30-50 platelets. By 12 hours the numbers of platelets had risen to approach the pre-treatment level. Giving two doses of 8,000 units within the space of 12 hours resulted in severe prolonged thrombocytopenia and in patient 2 (above) resulted in a decision to discontinue therapy with animal material.

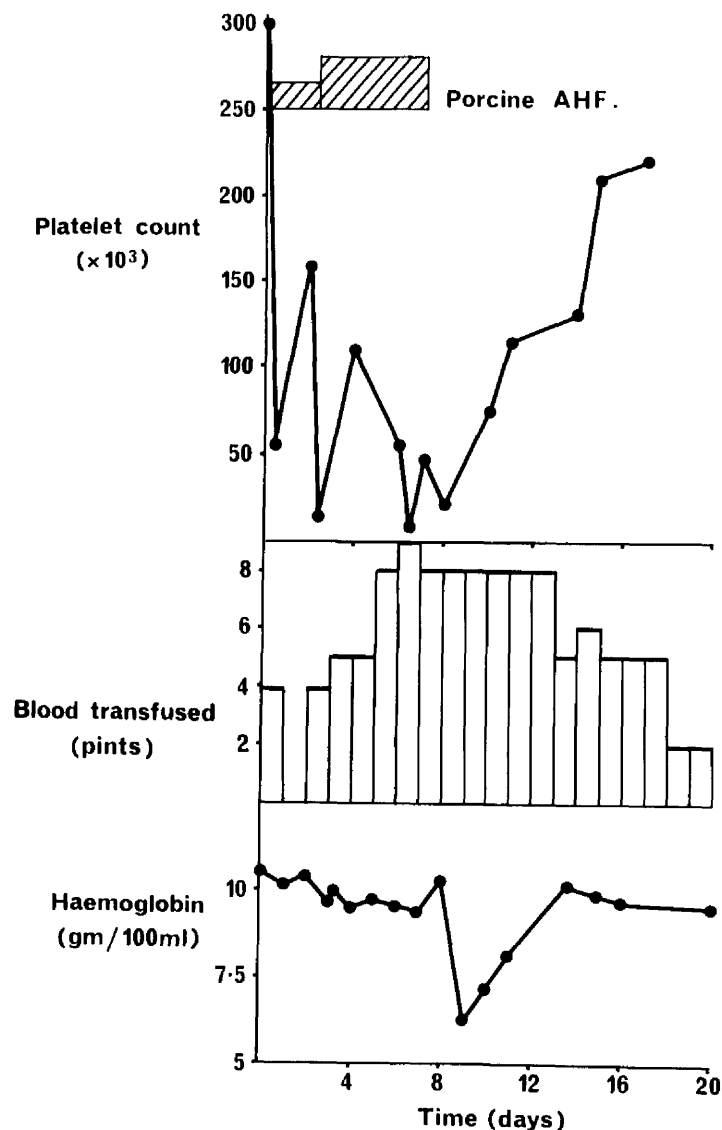


Fig. 34 Effect on platelet count, haemoglobin and the blood transfusion requirements of the infusion of porcine AHF. 8,000 units were given for the first two days and 16,000 units were given for the next three days. Severe thrombocytopenia produced an increase in gastrointestinal blood loss with a fall in haemoglobin despite a rise in the rate of blood transfusion. This patient had a high titre inhibitor and did not have a rise in plasma AHF after the porcine infusions.

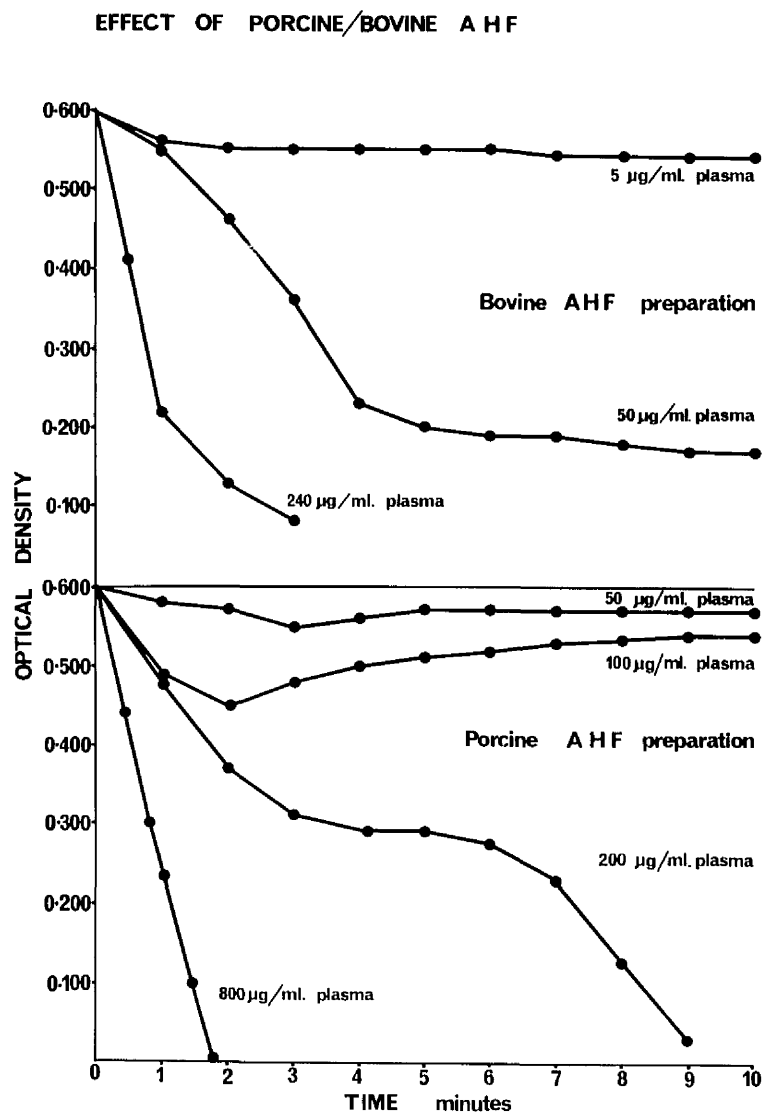


Fig. 35 Aggregation of human platelets by different concentrations of commercial porcine and bovine AHF preparations. In this system a fall in optical density indicates platelet aggregation. Irreversible aggregation of platelets was produced by bovine AHF at a concentration of 50 $\mu\text{g/ml}$ and by porcine AHF at 200 $\mu\text{g/ml}$. This would suggest that bovine AHF has, weight for weight, about four times the aggregating-activity of porcine AHF preparations. At certain concentrations platelet aggregation is reversible.

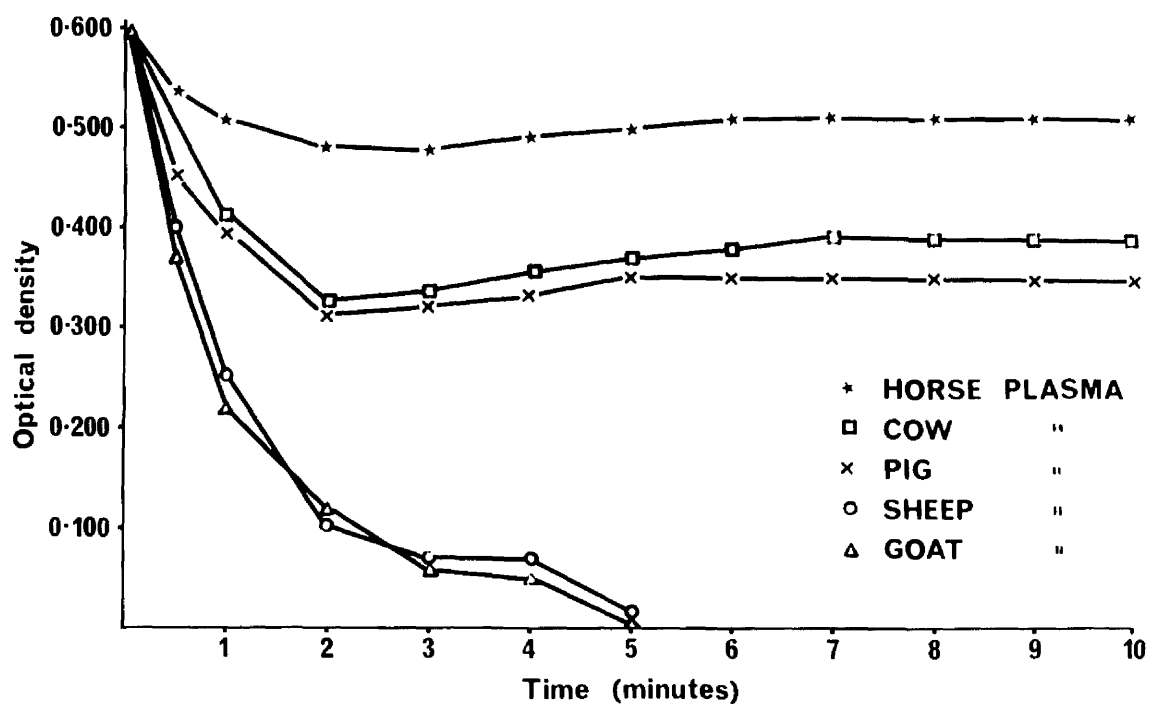


Fig. 36 Aggregation of human platelets by various mammalian plasmas. Aliquots of 0.1 ml of animal plasma were added to 2 ml of human platelet rich plasma. Rapid irreversible aggregation was produced by sheep and goat plasma and reversible platelet aggregation by cow, pig and horse plasma.

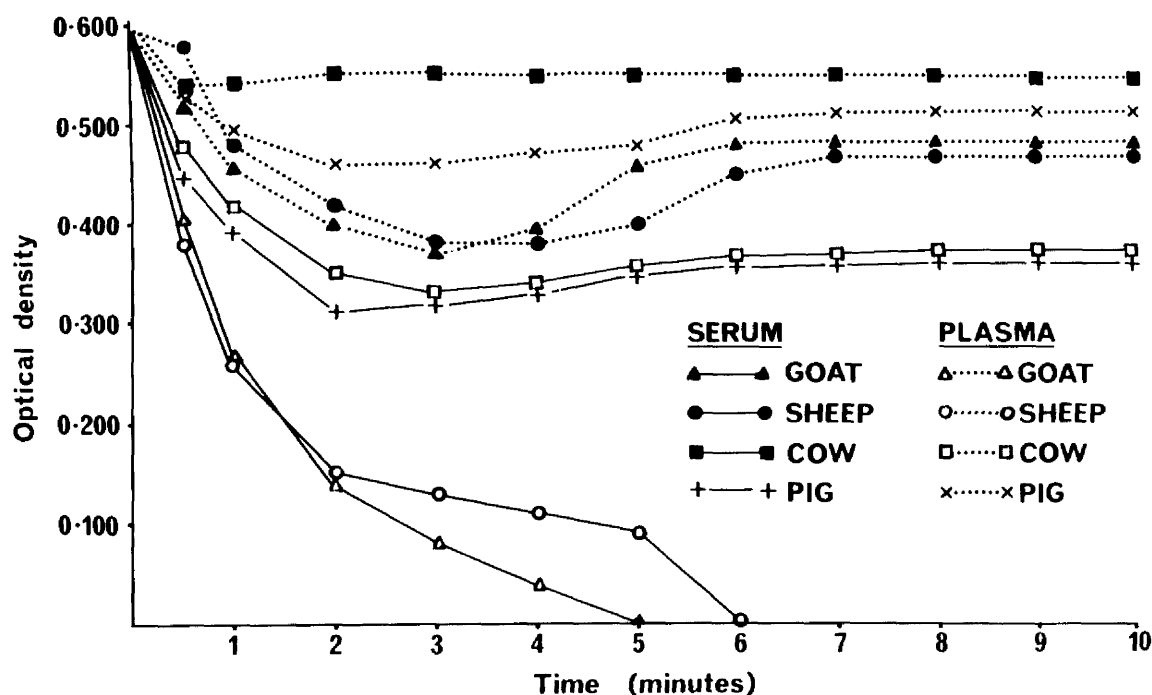


Fig. 37 Comparison of plasma and serum from various animal plasmas. in the aggregation of human platelets. Serum was obtained by clotting the plasma samples with the addition of calcium chloride (0.025 M) and then incubated at 37°C for three hours to remove any residual thrombin. Clotting of the plasma resulted in diminution of the aggregating activity in all cases, suggesting that the aggregating activity lay in the fibrinogen fraction or some closely related fraction.

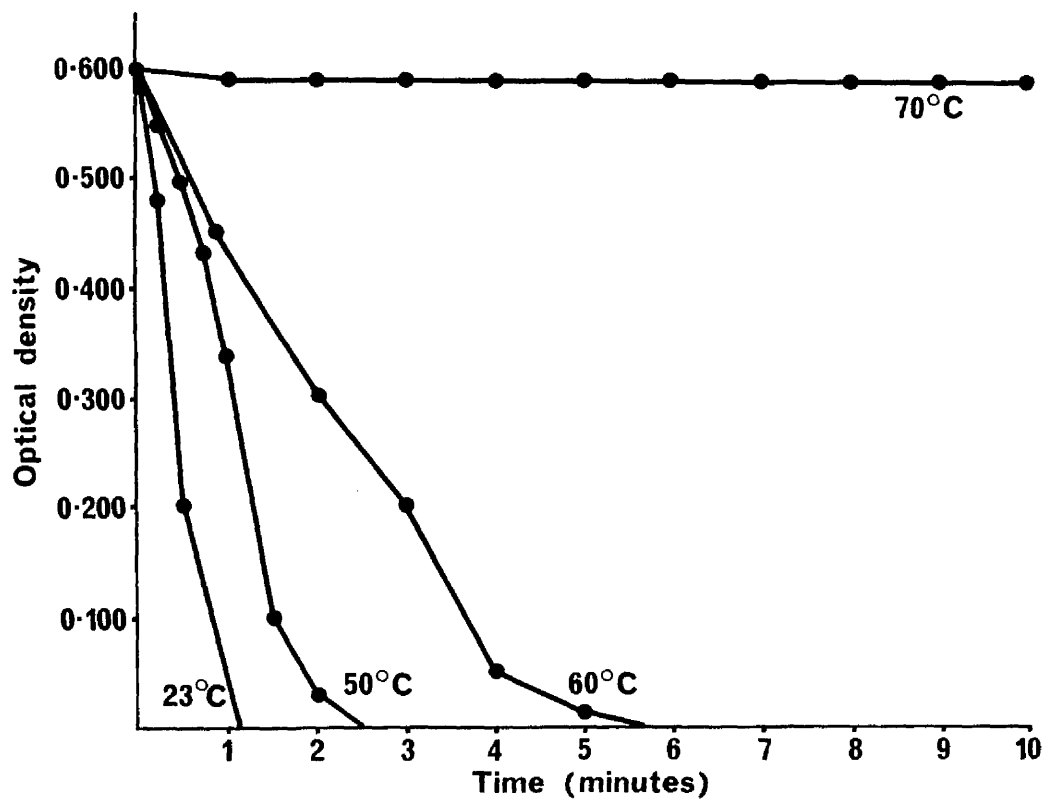
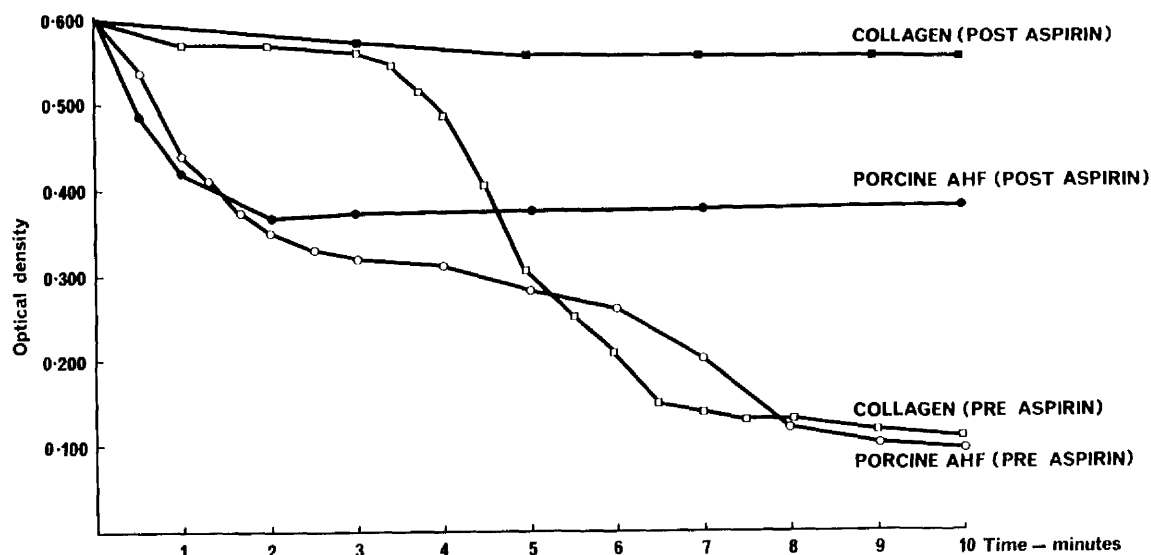
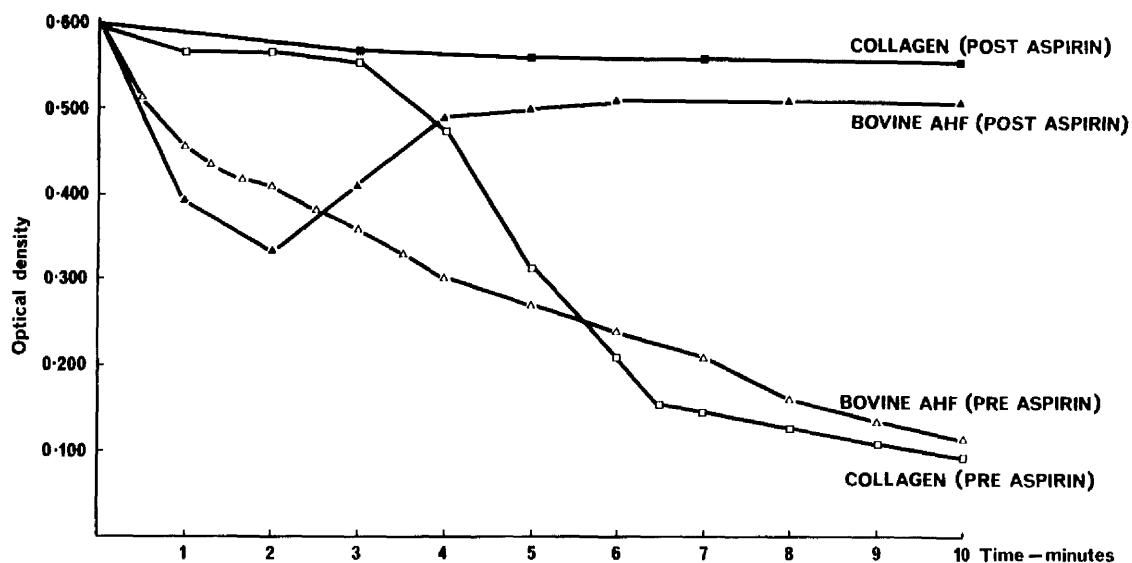


Fig. 38 Progressive inhibition of the platelet aggregating agent with rise in temperature. The effect on porcine AHF is shown.



a



b

Fig. 39 (a) and (b) The effect of ingestion of acetylsalicylic acid on aggregation of human platelets by AHF preparation showing inhibition of the second phase of aggregation after the ingestion of aspirin. (a) is porcine AHF, (b) is bovine AHF.

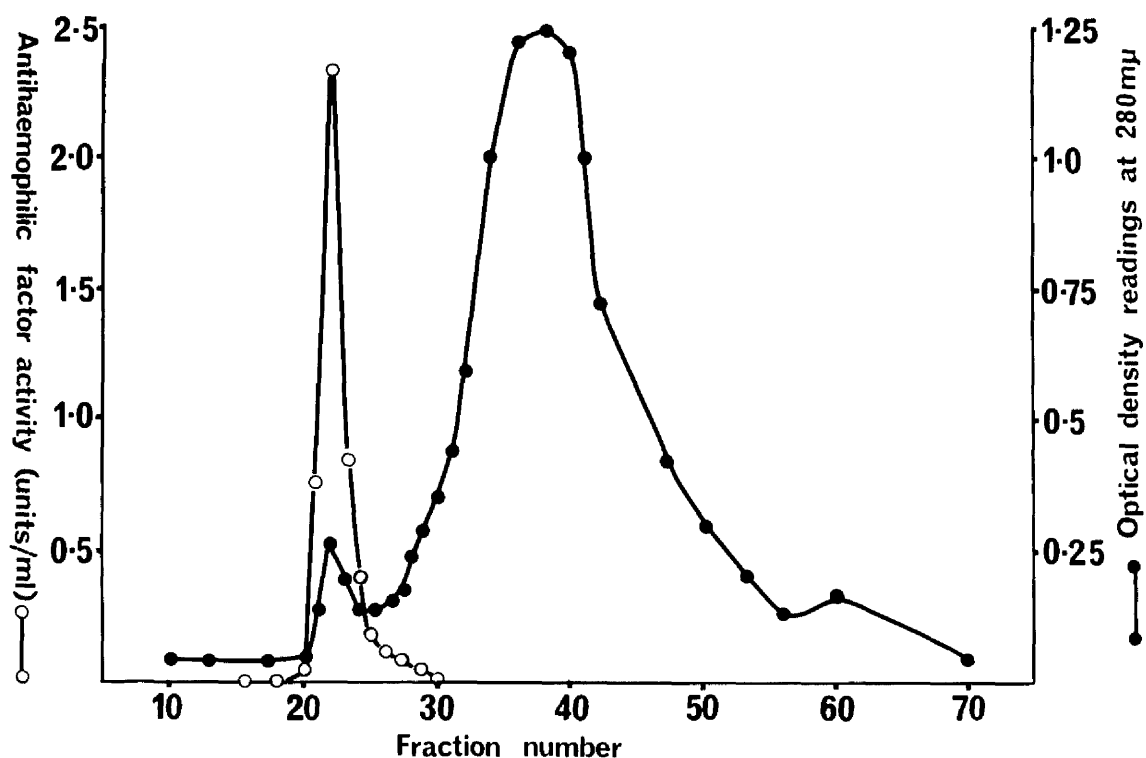


Fig. 40 (a) Gel filtration of the commercial preparations of porcine AHF on Sepharose 4B. This shows that there are two main protein peaks. The second peak consists of fibrinogen alone. The first contains all the AHF activity and the aggregating agent which have coincidental peaks of activity.

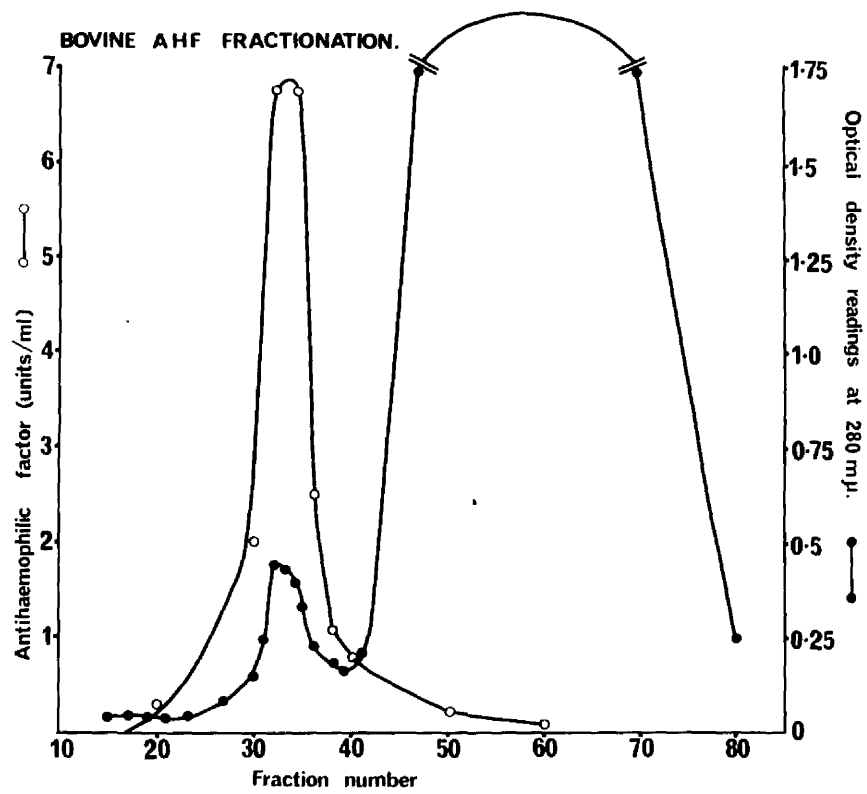


Fig. 40 (b) Gel filtration of bovine AHF on Sepharose 4B



Fig. 41 Polyacrilimide disc gel electrophoresis of a preparation of porcine AHF prepared by gel filtration and then concentrated x 100 by pressure dialysis is shown on the right. There is a faint band which lies in the same position as the purified porcine fibrinogen (left).

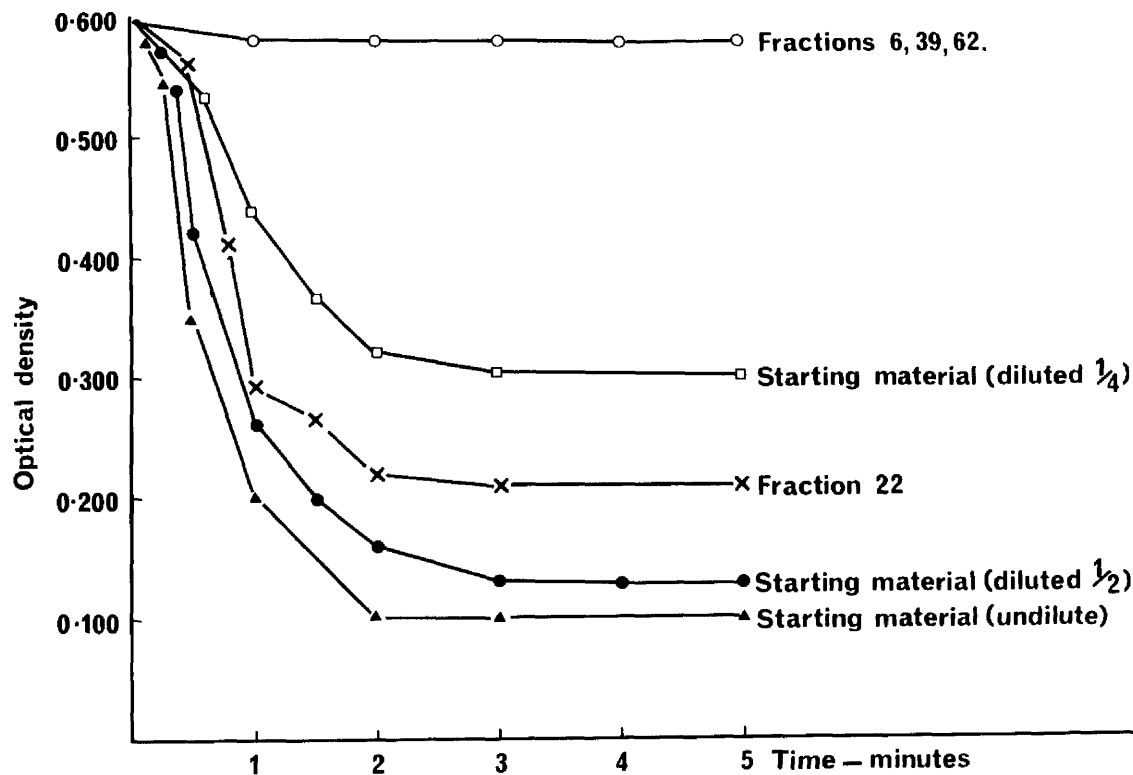


Fig 42 (a) Aggregation of human platelet rich plasma by samples of eluate from the Sepharose 4B columns shown in Fig. 40 (a). This shows that aggregating activity is found only in the samples of eluate from the first protein peaks i. e. the AHF. immunodiffusion.

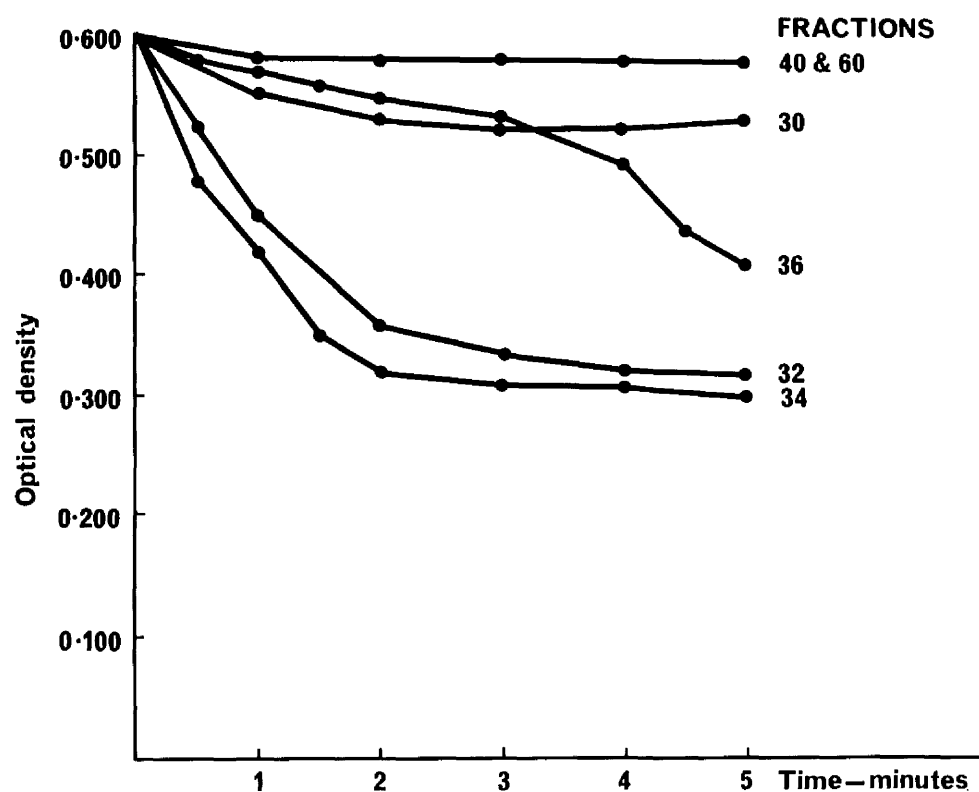


Fig. 42 (b) Aggregation of human platelet rich plasma by samples of eluate from column shown in fig. 40 (b).

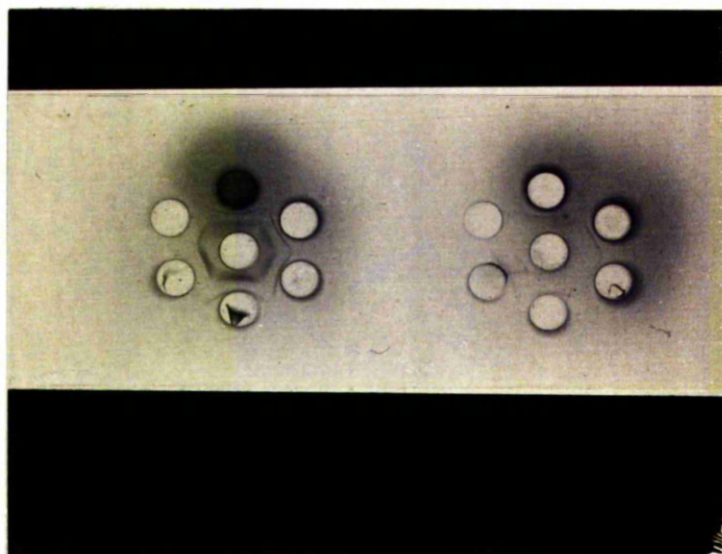


Fig. 43 Immunodiffusion slide with antibody in the central wells and doubling dilutions of crude porcine AHF in the peripheral wells. On the left is the crude rabbit antibody and this shows two lines against crude AHF and on the right, the antibody, which has been adsorbed with purified fibrinogen, shows a single line against the crude AHF preparation. This antibody preparation gives no lines against purified fibrinogen. It also rapidly destroys AHF activity when incubated with AHF at 37°C.

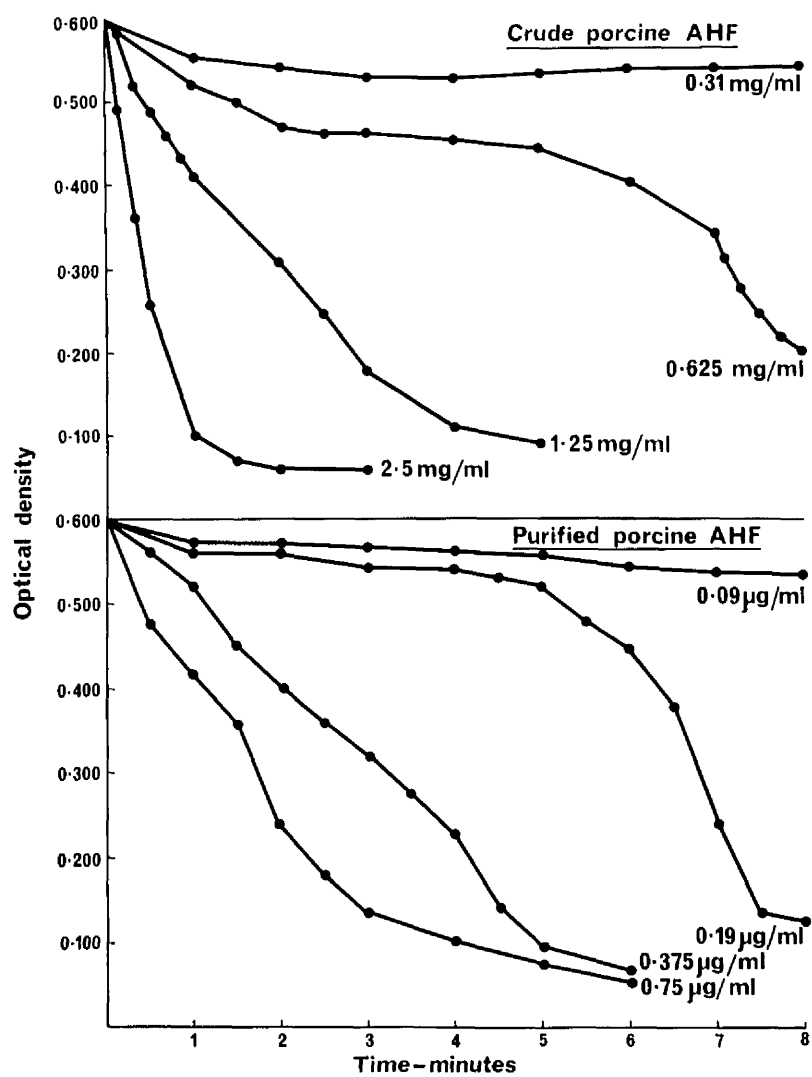


Fig. 44 Comparison of platelet aggregation between the purified porcine AHF material and the impure AHF. The pattern of platelet aggregation is similar with both preparations.

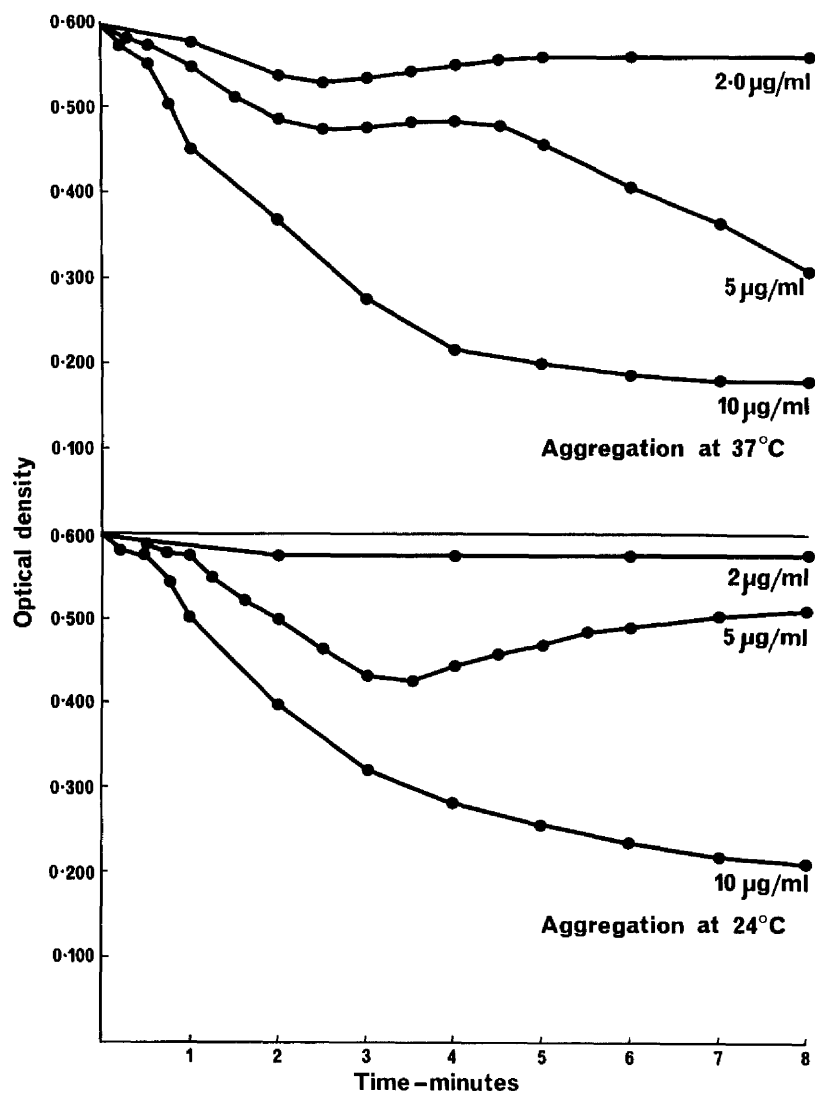


Fig. 45 Comparison of aggregation of human platelets by purified AHF at 24°C and at 37°C. It is apparent that at 37°C the pattern of platelet aggregation is different at some concentrations of AHF, from that at room temperature. Using a concentration of 5 µg/ml of purified porcine AHF there is a secondary phase of platelet aggregation which is irreversible whereas at 24°C the aggregation is reversible.

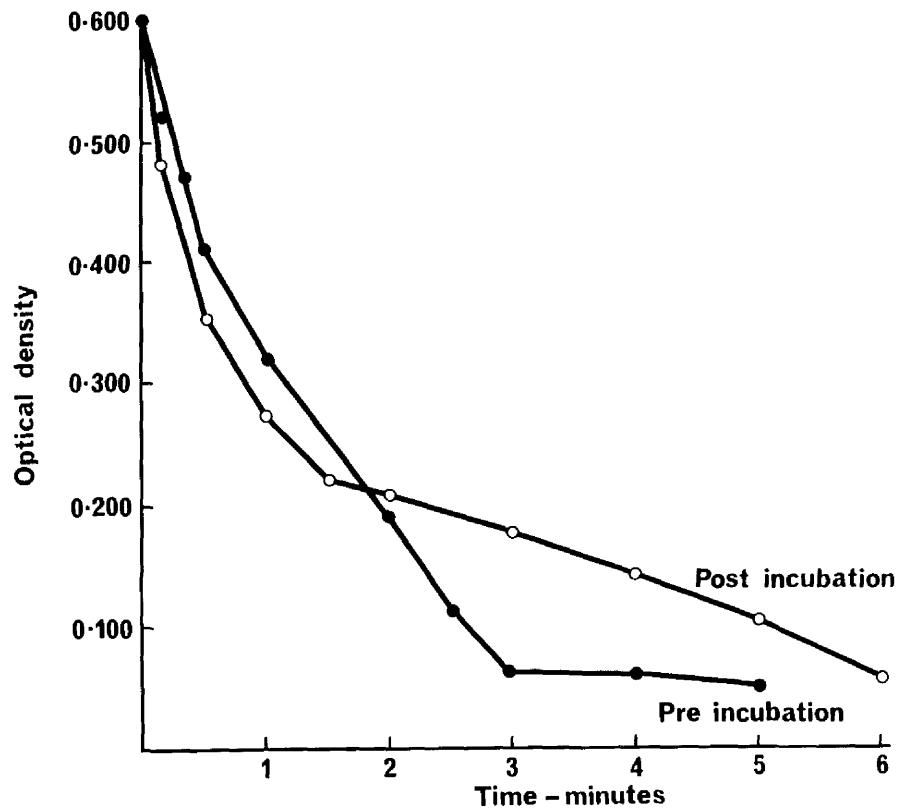


Fig 46 Aggregation of human platelets by porcine AHF before and after incubation with a spontaneous inhibitor from a haemophilic patient. The starting level of AHF activity was 2.8 units/ml. This AHF activity was totally destroyed when incubated with the inhibitor. No change occurred in the aggregating activity.

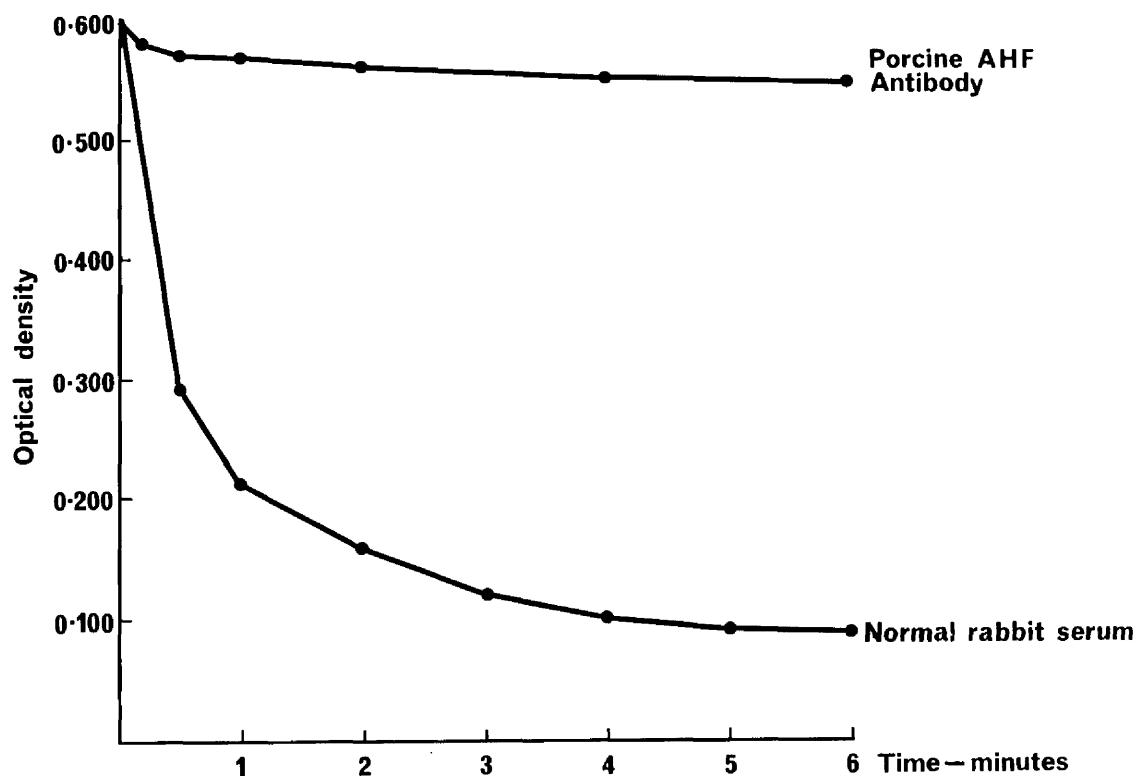


Fig. 47 Loss of aggregating activity after incubation of a monospecific rabbit antibody against AHF, with a purified fraction of porcine AHF. There is complete loss of AHF activity and of aggregating activity after incubation. There was no loss of aggregating activity when normal rabbit serum was used as a control.

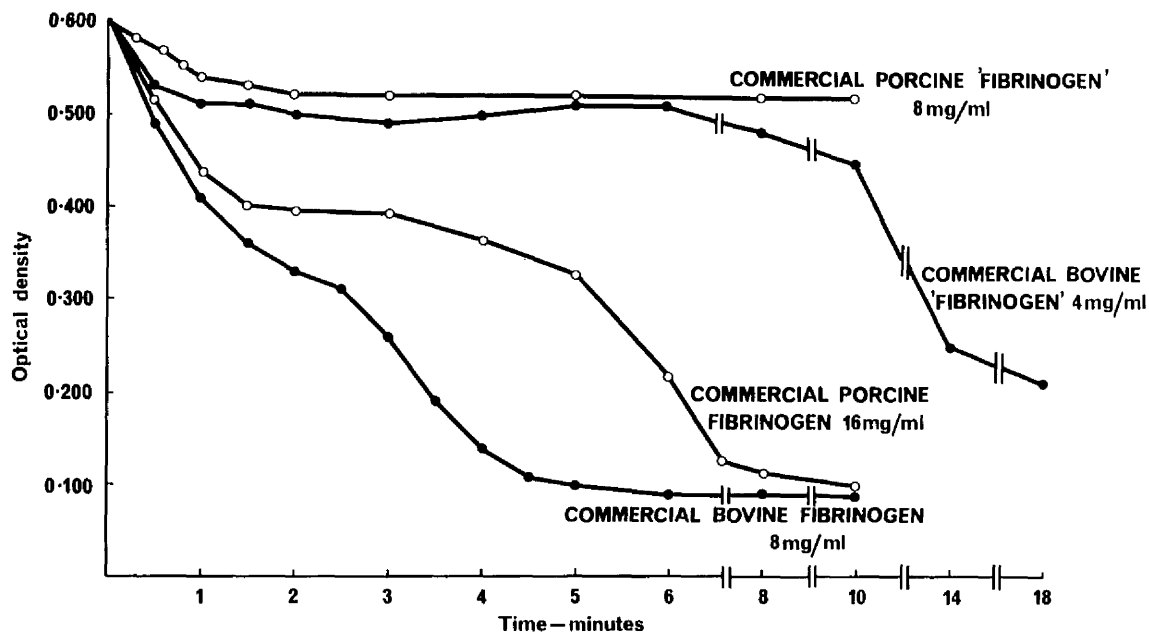


Fig.48 Aggregation of human platelets by commercial preparations of bovine and porcine fibrinogen. The pattern of aggregation is similar to that produced by AHF preparations. The aggregating activity in bovine fibrinogen seems to be between 2 - 4 times greater than the porcine fibrogen.

COMMERCIAL PORCINE FIBRINOGEN

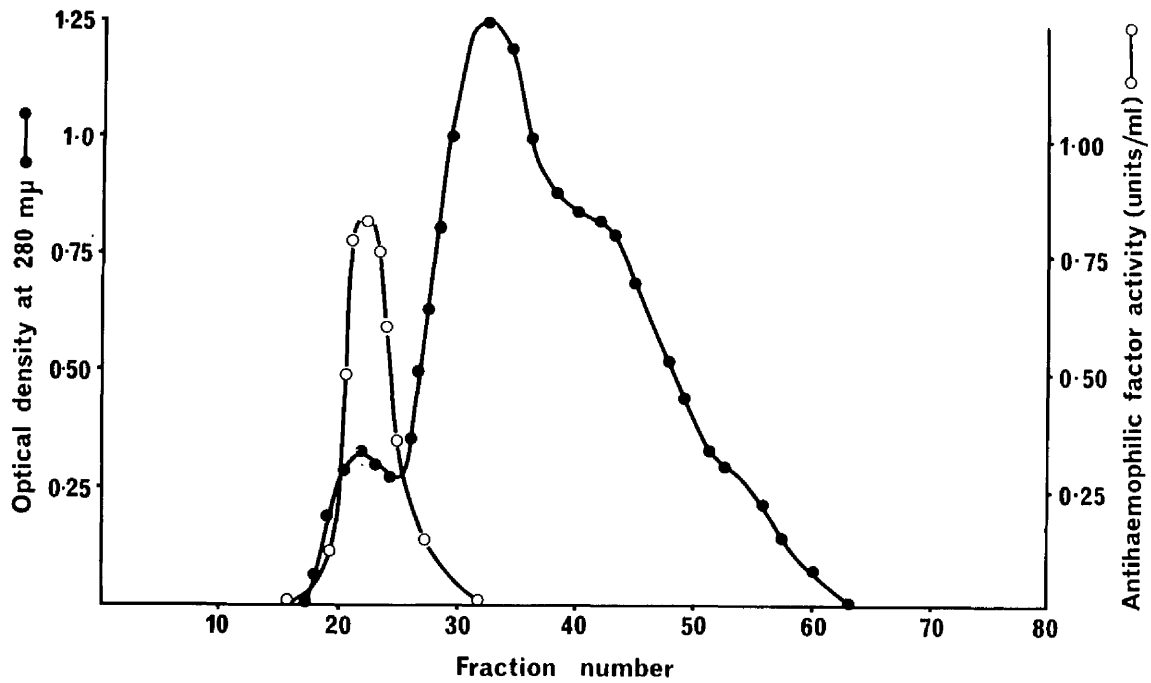


Fig. 49 (a) Gel filtration of porcine fibrinogen on Sepharose 4B. The pattern of separation is identical to that produced from the commercial AHF preparations. Platelet aggregating activity is localised to the peaks of AHF activity which contaminates these preparations.

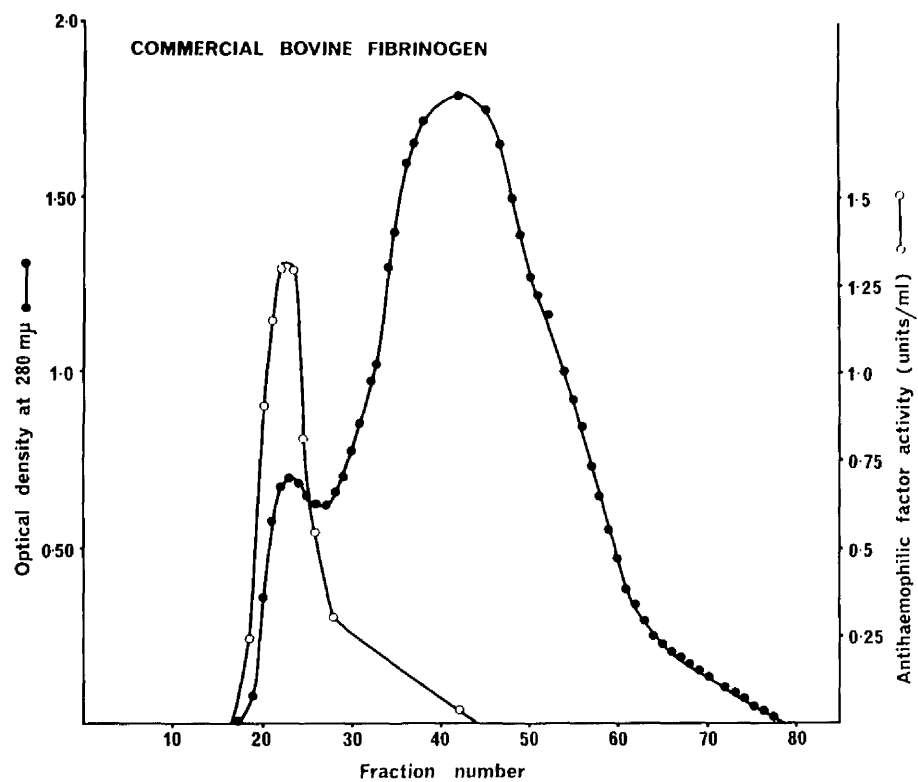


Fig. 49 (b) Gel filtration of bovine fibrinogen on Sepharose 4B.

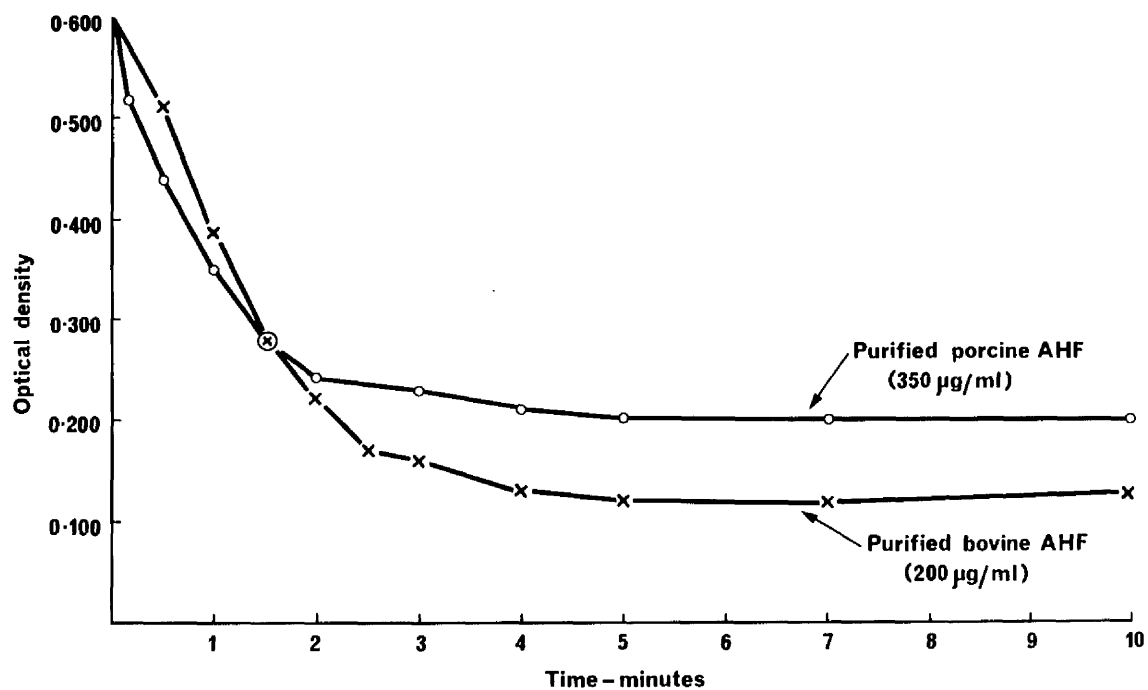


Fig. 50 Aggregation of gel filtered human platelets by purified bovine and porcine AHF. Comparison with previous figures will show that production of irreversible platelet aggregation requires a much higher concentration of the aggregating agent when "washed" platelets are used.

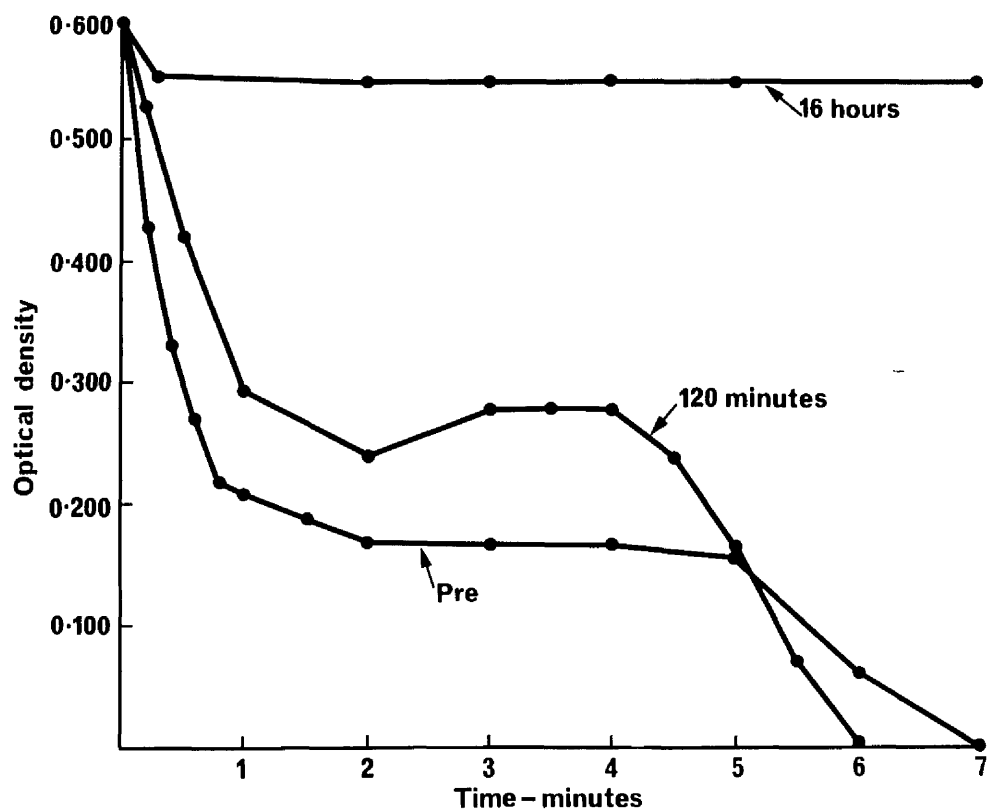


Fig. 51 (a) The effect of incubation of plasmin on aggregation produced by porcine AHF. There is progressive loss of AHF activity and also of the aggregating activity.

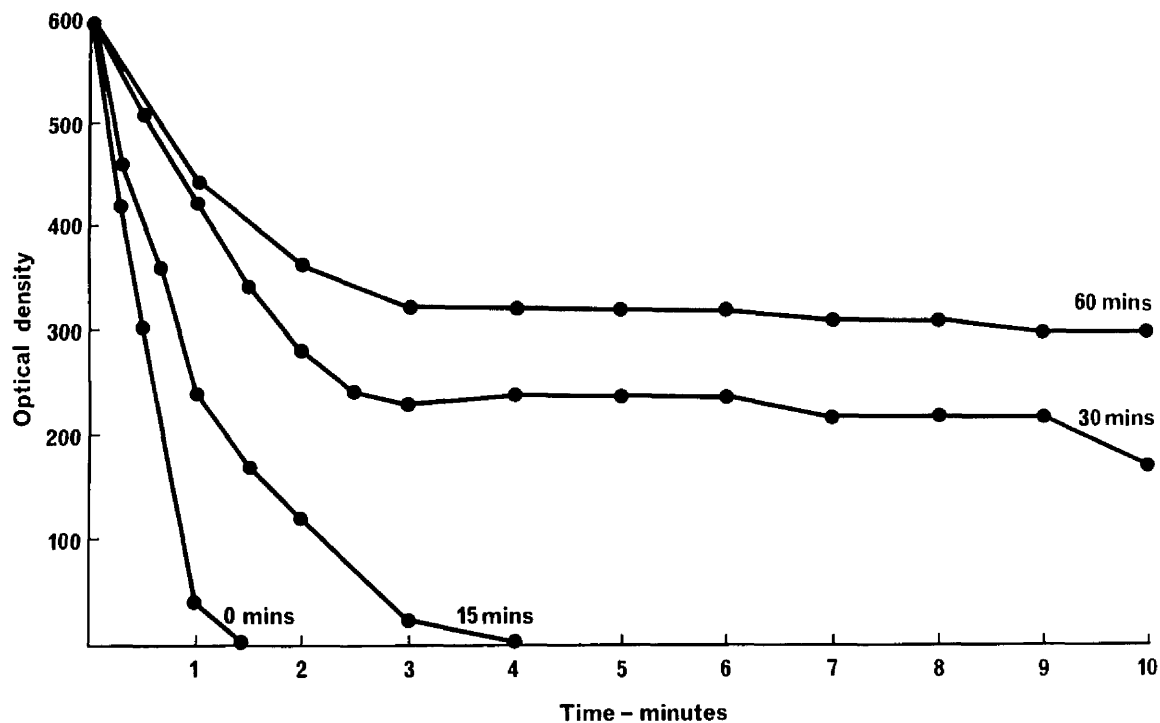


Fig. 51 (b) The effect of incubation of plasmin on aggregation produced by bovine AHF. There is progressive loss of AHF activity and also of the aggregating activity.

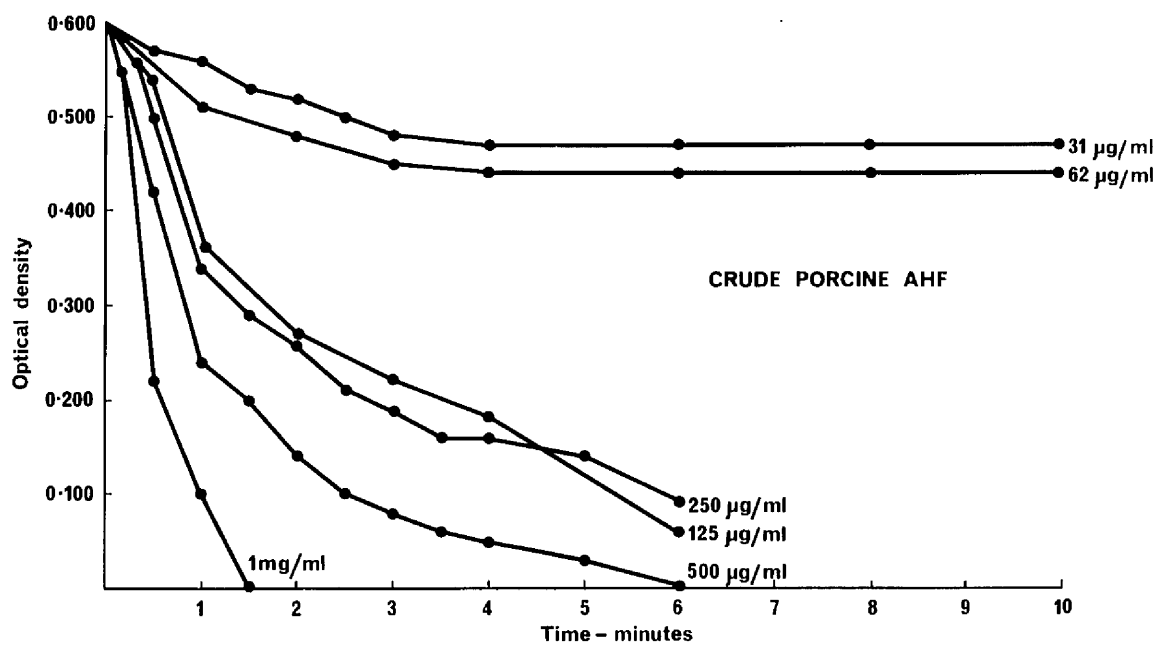


Fig. 52 (a) Aggregation of guinea pig platelets by porcine AHF.

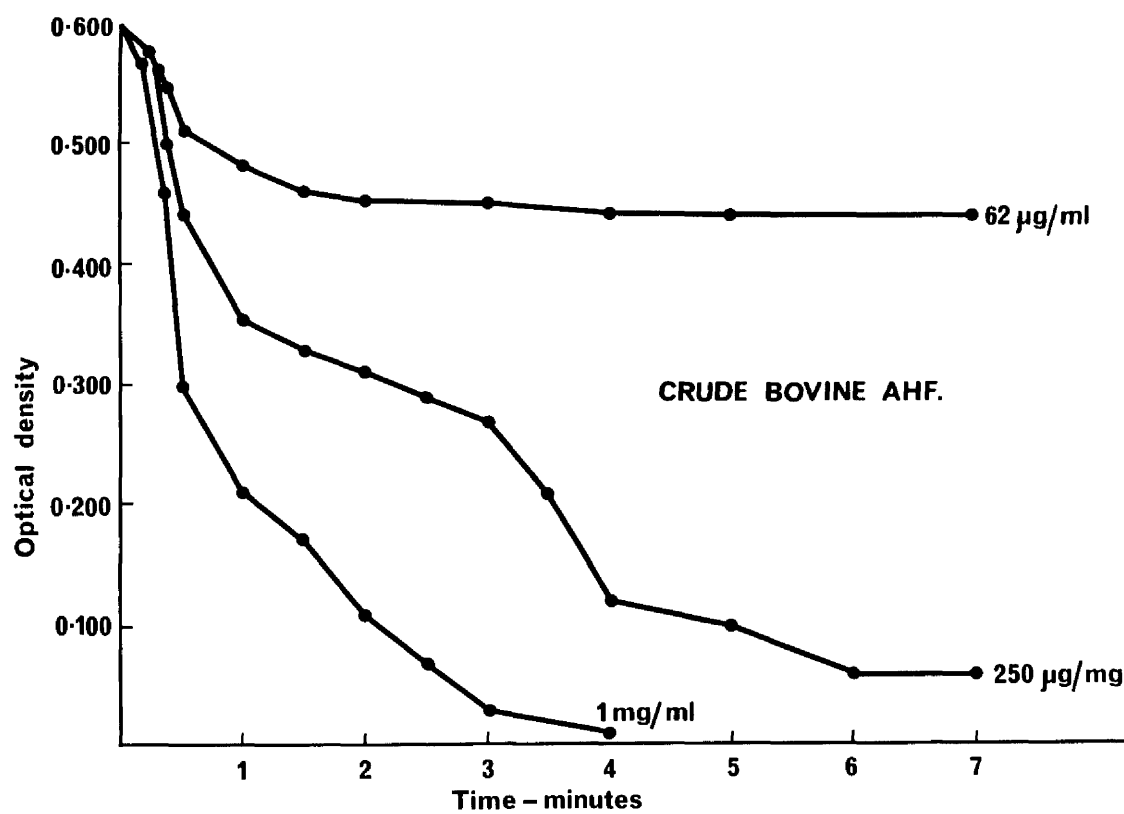


Fig. 52 (b) Aggregation of guinea pig platelets by bovine AHF.

APPENDIX 2

TABLES

TABLE 1 Radioactive scan scores in the knee joints of haemophilic patients with acute haemarthrosis

Patient number	Age	Severity	Incidence of bleeding score		'Signs' score		Scan	
			Right	Left	Right	Left	Right	Left
1	24	Severe	3	3	11* : 4		3	0
2	17	Severe	3	3	4 : 12*		1	3
3	14	Severe	3	3	0 : 8*		0	3
4	22	Severe	3	3	3 : 11*		1	3
5	30	Severe	3	3	4 : 12*		1	3
6	60	Severe	3	3	12* : 4		3	2

Shows clinical and radioactive scan details of seven severely affected haemophilic patients with acute haemarthrosis. All patients had sustained many previous bleeds into their knee joints and all had chronic joint changes on the X-ray

* Denotes the joint with the acute haemarthrosis

TABLE 2 Serial radioactive scan scores in a patient with a resolving acute haemarthrosis of left knee (Patient no. 4)

Date	'Signs' score		Scan score	
	Right	Left	Right	Left
1) Acute haemarthrosis 10.12.70	3 +	11*	1	3
2) 22.12.70	3	0	1	3
3) 14. 1.71	3	0	1	3
4) 18. 3.71	3	0	1	2
5) Acute haemarthrosis 13. 4.71	3	11*	1	3

Shows the rapid clinical response to AHF therapy with return of the 'signs' score to zero within 48 hours with only gradual improvement of the scan over twelve weeks. Progress complicated by a recurrence of the acute haemarthrosis in the left knee.

* Denotes joint with acute haemarthrosis

~~+~~ The right knee joint is the site of degenerative changes both clinically and radiologically

TABLE 3 Radioactive scan scores in two patients,
before and after an acute haemarthrosis

	'Signs' score		Scan score	
	Right	Left	Right	Left
Patient 2. 26.10.70	4	: 4	1	: 1
Acute haemarthrosis 6.5.71	4	: 12*	1	: 3
Patient 3. 10.11.70	4	: 4	1	: 1
Acute haemarthrosis 20.5.71	4	: 12*	1	: 3

Both patients had severe haemophilia with multiple previous episodes of bleeding into the knee joints and chronic arthritic changes as judged clinically and radio-
logically. Both have a baseline abnormality in the radioactive scans which is due to the chronic arthritis and both show a change in 'signs' and scan scores with the onset of the acute haemarthrosis.

* Acute haemarthrosis

TABLE 4 Clinical and radioactive scan scores in patients
with subacute haemophilic joint disease

Patient number	Age	Severity	Incidence of bleeding score		'Signs' score		Scan score	
			Right	Left	Right	Left	Right	Left
7	14	Moder- ate	0	1	0	9	0	3
8*	21	Severe	3	2	6	1	3	0

In this table subacute joint disease is defined as a swollen, non-tender joint which had been present for at least eight weeks. There had been no recent history suggestive of an acute haemarthrosis. The clinical signs underwent resolution with appropriate plasma concentrates.

* Patient has Christmas disease

TABLE 5 Clinical and radioactive scan scores in patients with chronic haemophilic joint disease

Patient number	Age	Severity	Incidence of bleeding score		'Signs' score		Scan score	
			Right	Left	Right	Left	Right	Left
9	24	Severe	1	: 3	0	: 4	2	: 3
10	25	Moder- ate	2	: 0	4	: 0	2	: 0
11	21	Moder- ate	1	: 0	1	: 0	2	: 2
12	19	Severe	3	: 3	1	: 2	3	: 3
13	12	Severe	3	: 3	0	: 1	3	: 3
14	48	Moder- ate	2	: 2	2	: 2	2	: 1
15	24	Severe	3	: 3	2	: 4	2	: 2
16	42	Severe	3	: 3	2	: 3	3	: 1
17	51	Severe	3	: 2	6	: 6	3	: 1
18*	32	Moder- ate	2	: 2	0	: 1	1	: 1
19	38	Severe	3	: 3	2	: 4	2	: 1
20	14	Severe	1	: 3	2	: 3	2	: 2
21	29	Severe	2	: 3	0	: 4	0	: 3
22	19	Severe	2	: 3	3	: 5	3	: 3
23*	12	Severe	2	: 0	3	: 0	3	: 3

All patients in this group had evidence clinically of chronic haemophilic joint disease in one or both knee joints.

* Patient had Christmas disease

TABLE 6 Radioactive scan scores in patients with haemophilia
but no apparent joint disease

Patient number	Age	Severity	Incidence of bleeding score		'Signs' score		Scan score	
			Right	Left	Right	Left	Right	Left
24	49	Mild	0	:	0	0	:	0
25	16	Mild	0	:	0	0	:	0
26	50	Mild	0	:	0	0	:	0
27	11	Mild	0	:	0	0	:	2
28	11	Mild	0	:	0	0	:	3
29	14	Mild	0	:	0	0	:	3
30	35	Mild	0	:	0	0	:	0
31	52	Moder- ate	0	:	0	0	:	0
32	31	Mild	0	:	0	0	:	0
33	14	Mild	0	:	0	0	:	0
34	16	Mild	0	:	0	0	:	0
35	34	Mild	0	:	0	0	:	0

None of the patients in this group had sustained previous bleeding into the knee joints and none had clinical evidence of arthritis. Radiology of all these joints showed no abnormality.

TABLE 7 Clinical, thermographic and radioactive scan scores in patients with acute and chronic haemophilic joint disease and in haemophilic patients with no apparent joint disease

Type of joint disease		Age	Severity	'Signs' score		Incidence score		Thermograph score		Scan score	
				Right	Left	Right	Left	Right	Left	Right	Left
Acute	1	22	Severe	11	3	3	3	3	0	3	0
	2	17	Severe	4	12	3	3	0	2	1	3
	3	60	Severe	12	4	3	3	3	2	3	2
	4	21*	Severe	7	0	3	2	3	0	3	0
	5	14	Severe	0	7	0	1	2	3	0	3
Chronic	6	12	Severe	0	1	3	3	0	1	3	3
	7	21	Moderate	1	0	1	0	1	0	2	2
	8	38	Severe	2	4	3	3	3	2	2	1
	9	51	Severe	6	6	3	2	2	1	3	1
	10	18	Severe	3	5	2	3	2	3	3	3
	11	22	Moderate	1	0	1	0	2	0	2	2
No joint disease	12	13	Mild	0	0	0	0	1	0	-	-
	13	13	Mild	0	0	0	0	1	1	0	0
	14	10	Mild	0	0	0	0	1	1	3	3

* Patient had Christmas disease

TABLE 8 Addendum on nomenclature of enzymes

Creatine kinase	CK	adenosine triphosphate creatine phosphotransferase	2.7.3.	2
Lactate dehydrogenase	LDH	L-lactate: NAD oxidoreductase	1.1.1.	27
Aspartate transaminase	GOT	L-aspartate: 2 oxoglutarate aminotransferase	2.6.1.	1
Alanine transaminase	GPT	L-alanine: 2 oxoglutarate amino transferase	2.6.1.	2
Aldolase		ketose-1-phosphate aldehyde kinase	4.1.2.	7

TABLE 3 Serum enzyme values in a control haemophilic population

Patients studied	Creatine kinase	Lactate dehydrogenase	Aspartate aminotransferase	Alanine aminotransferase
Control haemophiliac group (mean \pm S.D.)	32.7 \pm 25.2	271 \pm 102.4	18.6 \pm 12.9	14.3 \pm 13.7
Normal values for hospital population	0 - 100	115 - 465	9 - 35	5 - 26

All values in IU / litre

Normal levels of creatine kinase, lactate dehydrogenase, aspartate and alanine aminotransferases in a control group of hospitalised patients with haemophilia and Christmas disease. No patient had clinical evidence of muscle bleeding. There is no significant difference between this group and the normal non-haemophilic controls.

TABLE 10 Clinical data of patients with muscle haematomata

Patient number	Age (years)	Grade of defect	AHF or CF level (units per 100 ml)	Site of haematoma	Clinical size	Cause
1	15	Severe	0	Bilateral thigh	Massive	Automobile accident
2	15	Severe	0	Thigh	Massive	Intramuscular injection
3	15	Severe*	0	Thigh	Massive	Traumatic
4	46	Moderate	3	Buttock	Moderate	Traumatic
5	21	Severe	0	Buttock	Moderate	Traumatic
6	42	Severe	0	Thigh +	Small	Traumatic
7	34	Severe	0	Thigh	Small	Spontaneous
8	24	Severe	0	Deltoid +	Small	Spontaneous
9	13	Severe	0	Buttock	Small	Intramuscular injection
10	20	Severe*	0	Diagnostic problem	-	-

* Patient had Christmas disease

+ Associated with acute haemarthrosis of joint

TABLE 11 Serum enzyme levels in patients with muscle haematoma

Patient number	Creatine kinase	Lactate dehydrogenase	Aspartate aminotransferase	Alanine aminotransferase	Aldolase
1	17,400	2100	348	88	-
2	575	1020*	26	15	4.0
3	330	474*	27	11	9.4
4	568	420*	12	10	-
5	196	380	18	11	-
6	167	410*	14	12	-
7	112	335*	15	11	-
8	102	250	13	13	4.85
9	125	415	23	39	-
10	177	320*	11	10	-

* Electrophoresis of LDH 1 > 2 > 3 > 5 > 4

All patients had elevation of CK, the peak level of which bore a crude positive correlation to the clinical size of the haematoma. All patients showed the same pattern of change in CK, the baseline starting at a low level with a significant rise by 24 hours and the peak level occurring between 36 and 96 hours.

TABLE 12 Clinical details of patients undergoing ultra-
sonography of muscle haematomata

Patient	Disease	Severity	Age	Haematoma	
				Site	Size
1	Haemophilia	Severe	37	Calf	Massive
2	Haemophilia	Severe	16	Thigh	Massive
3	Haemophilia	Severe	23	Calf	Moderate
4	Haemophilia	Severe	22	Buttock	Massive
5	Circulating anticoagulant	-	58	Thigh	Massive
6	Christmas disease	Severe	25	Calf	Moderate
7	Haemophilia	Severe	14	Thigh	Moderate

TABLE 13 Clinical data of patients receiving AMCA or placebo

	PLACEBO	AMCA
Number of episodes of extraction	16	16
Number of patients	14	14
Number with haemophilia	9	11
Number with Christmas disease	5	3
Level of plasma factor (mean, per cent) (range)	4.5 (0 - 22)	5 (0 - 23)
Clinical severity: Severe	7	3
Moderate	6	5
Mild	1	1

Patients are equally divided between the test and placebo groups with regard to number of episodes of extraction, disease, severity and assay of levels of coagulation factors.

TABLE 14 Results in the AMCA treated and placebo groups after dental extraction

	PLACEBO	AMCA
Number of roots extracted	(mean) 5.5 (range) (2-12)	6.9 (2-22)
Blood loss per patient (ml)	(mean) 84.1 (range) (4-323)	61.2 (1-749)
Blood lost per root extracted (ml)	(mean) 15.3 (range) (0.5-64)	8.9 (0.5-38.6)
Units of replacement therapy per root extracted (units)	(mean) 617 (range) (0-15, 800)	30 and 65 in two patients
Fall in haemoglobin (Gms per 100 ml)	(mean) 1.4	0.3
Fall in haematocrit (per cent)	(mean) 5.0	0.9

Haemophilic patients treated with AMCA show a significant reduction in blood loss ($0.01 < p < 0.025$) and a highly significant ($p < 0.001$) reduction in plasma requirements.

TABLE 15 Clinical data of 15 patients with haemophilia or Christmas disease who have had no history of dyspepsia associated with gastrointestinal bleeding

Patient number	Severity	Age at first bleed	Number of episodes	Association with salicylates or alcohol	Follow up period from first gastrointestinal bleed (years)	Diagnosis	Medical or surgical treatment
1	Severe	50	1	Salicylates	9	Salicylate induced melaena	Medical
4	Severe	6	2	No	16	No diagnosis	Medical
5	Severe	27	1	No	3 months	Duodenal haematoma	Medical
6	Mild	52	1	No	7	Chronic myeloid leukaemia	Medical
7	Severe	15	14	3 episodes associated with alcohol	7	D. U.	Surgical
8	Moderate	24	2	Salicylate ingested on 1st occasion	4	No diagnosis	Medical
10	Severe	50	1	No	11	No diagnosis	Medical
11	Severe	24	2	No	Patient died after 3 weeks	Multiple erosions of intestine	Medical

TABLE 15 (contd.) Clinical data of 15 patients with haemophilia or Christmas disease who have had no history of dyspepsia associated with gastrointestinal bleeding

Patient number	Severity	Age at first bleed	Number of episodes	Association with salicylates or alcohol	Follow up period from first gastro-intestinal bleed (years)	Diagnosis	Medical or surgical treatment
13	Severe	23	5	No	7	No diagnosis	Medical
16	Moderate	45	5	Salicylate ingested on 1st occasion	4	D. U.	Medical
18	Severe	63	4	No	3	No diagnosis	Medical
21	Severe	21	2	Alcohol ingested on 2nd occasion	11	No diagnosis	Medical
22	Severe	18	3	Salicylate taken on 3rd occasion	7	No diagnosis	Medical
30	Severe	27	1	No	3	Haematoma of ileum and volvulus	Surgical
32	Moderate	26	5	No	37	No diagnosis	Medical

TABLE 16 Clinical data of 17 patients with haemophilia or Christmas disease who have had dyspeptic symptoms associated with gastrointestinal bleeding

Patient number	Severity	Age at first bleed	Number of episodes	Association with salicylates or alcohol	Follow up period from first gastro-intestinal bleed (years)	Diagnosis	Medical or Surgical treatment
2	Moderate	34	2	No	5	Duodenal ulcer	Medical
3	Severe	26	6	Salicylates with 3. Al-cohol with 2	12	Duodenal ulcer	Medical
9	Severe	54	1	No	-	No diagnosis	Medical
12	Moderate	44	3	No	Barium meal positive before bleed	D. U.	Surgical
14	Moderate	47	2	Alcohol in 2nd	Barium meal positive before bleed	D. U.	Surgical
15	Moderate	31	1	No	-	No Diagnosis	Medical
17	Moderate	20	4	No	Barium meal positive before bleed	D. U.	Medical
19	Severe	16	3	No	Barium meal positive before bleed	G. U.	Medical

TABLE 16 (contd.) Clinical data of 17 patients with haemophilia or Christmas disease who have had dyspeptic symptoms associated with gastrointestinal bleeding

Patient number	Severity	Age at first bleed	Number of episodes	Association with salicylates or alcohol	Follow up period from first gastro-intestinal bleed (years)	Diagnosis	Medical or Surgical treatment
20	Moderate	41	15	No	15	D. U.	Medical
23	Moderate	23	1	No	1½	D. U.	Medical
24	Mild	21	3	No	Barium meal positive before bleed	D. U.	Medical
25	Severe	29	3	No	11	D. U.	Medical
26	Moderate	28	1	No	5	D. U.	Medical
27	Mild	17	3	Salicylates in 1st	5	D. U.	Medical
28	Mild	59	4	No	16	G. U.	Medical
29	Moderate	27	4	No	9	D. U.	Surgical
31	Mild	22	2	No	13	D. U. and G. U.	Surgical

TABLE 17 Comparison of the grade of defect
in the two groups of haemophilic
patients with gastrointestinal bleeding

Grade of defect	Severe	Moderate	Mild
Patients without dyspepsia	11*	3+	1
Patients with dyspepsia	4	9●	4

* Include 2 with Christmas disease

+ Include 1 with Christmas disease

● Include 1 with Christmas disease

There is a significant difference in the distribution of
the severity of the disease in the two groups of patients
($\chi^2 = 4.5$, $p < 0.05 > 0.02$).

TABLE 18 Clinical data, indication for surgery and outcome in patients with gastrointestinal bleeding who underwent surgery

Patient number	Diagnosis	Procedure	Indication for surgery	Comment
7	Duodenal ulcer	Pyloroplasty / vagotomy	Recurrent haematemesis	Further haematemesis Vagotomy incomplete
12	Duodenal ulcer	Pyloroplasty / vagotomy	Intractable dyspepsia	Symptom free for two years+
19	Gastric ulcer	Partial gastrectomy	Recurrent haematemesis	Two further haematemesis but symptoms free for two years
20	Duodenal ulcer	Pyloroplasty / vagotomy	Recurrent haematemesis	Mild dumping syndrome post-op.
29	Duodenal ulcer	Pyloroplasty / vagotomy	Intractable dyspepsia	Symptom free for six years
30	Ileal haematoma / volvulus	Resection of ileum	Intestinal obstruction	Symptom free for two years*
31	Duodenal ulcer / gastric ulcer	Partial gastrectomy / vagotomy	Intractable dyspepsia	Mild dumping syndrome

+ Developed serum hepatitis as a result of therapy at time of operation

* Developed an AHH inhibitor at the time of operation

All procedures were elective except for patient 30.

TABLE 19 Results of electroencephalography in 30 patients with haemophilia and Christmas disease

Clinical grade	Number of patients	Age range (years)	Antihaemophilic factor level	Abnormal E. E. G.
Severe	15	13 - 51	1 %	2 *
Moderate	8	13 - 46	1 - 5 %	2 †
Mild	7	11 - 54	5 - 20 %	1

There is no difference in the incidence of E. E. G. abnormalities in this group of patients from the general hospital population.

* Includes one patient with a subarachnoid haemorrhage in whom the E. E. G. reverted to normal with haemostatic treatment

† Includes one patient with long standing epilepsy

TABLE 20 Laboratory data in patients with clinical hepatitis

Patient number	Maximum values obtained during acute illness						Austl Antigen
	Bilirubin	Alkaline phosphatase	G. O. T.	G. P. T.	Protein	Albumin	
1	11.7	36	1020	880	8.3	4.8	Antigen* positive
2	7.0	97	180	140	7.9	2.5	Antigen* positive
3	9.3	19	2400	2900	8.0	4.5	Negative
4	24	41	1340	780	7.5	3.6	Negative
5	11	38	1400	1640	9.7	4.9	Negative

* Antigen cleared after three weeks

+ Antigen has been present since the hepatitis two years ago

All these patients had received blood or plasma products in the preceding six months.

TABLE 21 Clinical data of patients with anicteric hepatitis

Patient number	Maximum values obtained during acute illness							Antigen or antibody
	Bilirubin	Alkaline phosphatase	G.O.T.	G.P.T.	Total protein	Albumin	Globulin	
6	1.0	23	94	80	7.5	4.6	2.9	Negative
7	0.3	22	-	-	7.2	3.1	4.1	-
8	2.0	13	136	168	7.4	4.5	2.9	Negative
9a	2.0	14	150	340	7.4	3.5	3.9	Negative
b	1.3	19	430	420	7.7	3.8	3.8	Negative
10	0.9	12	15	124	8.3	4.6	3.7	Antigen+ positive
11a	0.7	40	132	244	-	-	-	Negative
b	1.2	39	55	105	-	-	-	Negative
12	0.7	15	58	132	7.4	4.5	2.9	Negative
13	0.4	13	120	136	7.3	4.9	2.4	Negative
14	1.4	18	79	112	8.3	5.0	4.3	Antibody* positive
15	0.4	14	13	68	8.6	4.8	3.8	Negative
16	1.1	13	60	70	7.0	4.1	2.9	Negative

+ Antibody became positive in this patient after two weeks

* Antibody became positive after onset of hepatitis but no antigen was found.

TABLE 22 Levels of coagulation factors in 1 unit (approximately 20 ml) of cryoprecipitate

Factor	Level (Mean \pm S.D.)
II	48 \pm 19 per cent
V	65 \pm 9 per cent
IX	52 \pm 6 per cent
VIII	130 \pm 79 units per pack
Fibrinogen	557 \pm 21 mg/100 ml
Plasminogen	2.8 \pm 0.25 c.u. /ml
Plasminogen activator	Nil

This shows the wide variation in level of AHF (FVIII) in individual packs of cryoprecipitate and this is one of the major disadvantages of its use. Fibrinogen is precipitated simultaneously with AHF and is the major protein constituent. During long continued administration of cryoprecipitate a state of hyperfibrinogenaemia appears due to the relatively long survival in the circulation.

TABLE 23 Incidence of blood group antibodies in
128 patients with haemophilia and
Christmas disease

Number of patients	Antibody
3	Anti - K
4	Anti - D
2	Anti - C + D + C
1	Anti - C
1	Anti - E

All patients except one had received multiple blood transfusions as well as plasma products before detection of the antibody. One patient developed an anti - D after infusion of cryoprecipitate alone.

APPENDIX 3

ADDITIONAL METHODS

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(1) Fibrinogen assay

The method described is that of Ratnoff and Menzie (1951) as modified by Alkjaersig (1960). In this assay fibrinogen is clotted with thrombin; the fibrin so formed is hydrolysed with sodium hydroxide and the tyrosine released is estimated colourimetrically. A constant proportion of tyrosine in the fibrinogen molecule is assumed (1:11.7).

In a 15 ml test tube are placed "0.2 ml" glass beads (diameter 0.15 mm), 6.0 ml saline, 0.1 ml thrombin solution, 100 N.I.H. units/ml, 0.2 ml, 2.5 per cent calcium chloride and 0.2 ml plasma. The tube is shaken but not inverted and the fibrin is caught up on the glass beads. After standing for one hour at 4°C the tube is shaken again and centrifuged for 10 minutes at 2,000 r.p.m. The glass beads and adherent fibrin are washed three times with saline. After final centrifugation and decantation of the washing fluid, 0.4 ml 10 per cent sodium hydroxide is added and the tube boiled in a water bath for 20 minutes. After cooling to room temperature, 0.6 ml 5 per cent trichloroacetic acid, 2 ml 0.5 N sodium hydroxide and 0.6 ml dilute (1:2)

Folin Ciocalteu reagent are added. After standing for 15 minutes for colour development, optical density at 650 m μ is read against a reagent blank. Readings are converted to fibrinogen concentration, mg/100 ml, from a standardised tyrosine curve.

Alkjaersig, N. (1960) in N.I.H. Conference on Thrombolytic Agents, eds. Roberts, H.R., Geraty, J.D., University of North Carolina Press, Chapel Hill, p. 316

Ratnoff, O.D., Menzie, C. (1951) 37, 316, Journal of Laboratory and Clinical Medicine

(2) Plasminogen assay

The method used was the caseinolytic assay of Remmert and Cohen (1949) as modified by Alkjaersig et al. (1959). Anti-plasmin is first destroyed by incubating the plasma with acid. The acid is neutralised with alkali and buffer, and streptokinase is then added to convert the plasminogen to plasmin. The plasmin so produced is assayed by a caseinolytic technique, the amount of tyrosine released from the casein being a measure of the amount of plasmin present.

The casein solution used is prepared by boiling for 20 minutes 25 gm casein ("Hammarsten" quality, Nutritional Bio-

chemicals Corporation, Cleveland, Ohio) in 500 ml phosphate buffer, 0.1 Molar, pH 7.6. The solution is filtered while hot and after cooling, the pH is readjusted to 7.6.

To 0.5 ml plasma is added 0.5 ml 1/6 N hydrochloric acid. After standing for 15 minutes at room temperature to destroy antiplasmin 0.5 ml 1/6 N sodium hydroxide is added, followed by 1.0 ml phosphate buffer, 0.1 Molar, pH 7.6, 0.5 ml streptokinase solution 2,000 units/ml. (Varidase-Lederle), and 2.0 ml 5 per cent casein solution. After addition of casein the assay mixture is incubated at 37°C for 62 minutes. At 2 minutes and 62 minutes 2 ml aliquots are taken and to each is added 2 ml 10 per cent trichloroacetic acid. After centrifugation (2,000 r.p.m. for 10 minutes) 1 ml of the supernatant is added to 5 ml 0.5 N sodium hydroxide and 1.5 ml 5 per cent trichloroacetic acid, followed by 1.5 ml dilute (1:2) Folin Ciocalteu reagent. After standing for 15 minutes for colour development, the optical density of the 62 minute sample is read at 650 mμ with the 2 minute sample as blank. Tyrosine release is read off a standard curve, one casein unit equals 180 mg tyrosine released in one hour.

Alkjaersig, N., Fletcher, A.P. and Sherry, S. (1959) Journal of Clinical Investigation, 33, 1086

Remmert, L.F. and Cohen, P. (1949) Journal of Biological Chemistry, 181, 431

(3) Euglobulin lysis

The fraction of plasma which precipitates in dilute solutions at pH 5.3 (the euglobulin fraction) contains plasminogen activator, plasminogen and fibrinogen, but antiplasmin is largely in the supernatant (Macfarlane and Pilling, 1946). If the euglobulin precipitate is resuspended and clotted with thrombin, much shorter lysis times are found than with the whole plasma from which the euglobulin precipitate was prepared (for a normal plasma, hours instead of days). The technique is therefore more convenient than whole plasma clot lysis time determination.

Method

The method used was that of Sherry et al. (1959). The test is set up within 30 minutes of withdrawal of the sample, which is stored at 4°C. To 7.4 ml acetic acid, pH 5.3, is added 0.6 ml plasma. After standing 10 minutes at 4°C, the precipitate is centrifuged down at 2,000 r.p.m. for 10 minutes in a refrigerated

centrifuge. The supernatant is poured off, the precipitate is dissolved in 0.7 ml barbitone buffer in 0.9 per cent saline, pH 7.4, and clotted with 0.1 ml thrombin, 10 N.I.H. units/ml. Time for complete clot lysis at 37°C is noted.

Calculation of results

Results are expressed in arbitrary units. It has been shown by Sherry and Alkjaersig (1957) that in fibrinolytic assays, activity is a direct function of the reciprocal of the lysis time, and accordingly a logarithmic plot of lysis times against units of activity shows a linear relationship. Euglobulin lysis times are expressed in terms of arbitrary units of activity derived from such a plot. A lysis time of 30 minutes is assigned an activity of one unit, and the values for other lysis times are obtained by dividing the lysis times observed into 30. Normal euglobulin lysis times are over 300 minutes (i. e. less than 0.1 unit) and if lysis has not taken place the test is discontinued at 300 minutes, the result being presented as "less than 0.1 unit".

Macfarlane, R.G. and Pilling, J. (1946), *Lancet*, 2, 562

Sherry, S. and Alkjaersig, N. (1957) Thrombosis et Diathesis Haemorrhagie (Stuttg.), 1, 264

Sherry, S., Lindemeyer, R.I., Fletcher, A.P. and Alkjaersig, N. (1959) Journal of Clinical Investigation, 38, 810

(4) Urokinase sensitivity test

The test was performed according to the method of McNicol and Douglas (1964). 0.2 ml of plasma, 0.32 ml of urokinase (500 μ /ml), 0.1 ml of thrombin (20 μ /ml) are added together in a 10 x 75 mm glass tube and allowed to clot. The time taken for dissolution of the clot is recorded. The time is then converted to arbitrary units, 1 unit of activity being equivalent to 10 minutes, 2 units are equivalent to 5 minutes and $\frac{1}{2}$ unit being equivalent to 20 minutes.

McNicol, G.P. and Douglas, A.S. (1964) in 'Recent Advances in Clinical Pathology', edited by Dyke, S.C.

(5) Assays for antihaemophilic factor and Christmas factor

The method described is that of Margolis (1958) as modified by Breckenridge and Ratnoff (1962).

- (a) Prepare a kaolin-phospholipid mixture by mixing 5 ml phospholipid with 50 mgm kaolin in a mechanical homogeniser. Using a 1 ml glass or plastic pipette, immediately pipette 0.1 ml of the kaolin-phospholipid mixture into as many 10 x 75 mm disposable glass tubes as will be needed. To do this, draw 1 ml of the mixture into the pipette and fill no more than six tubes; if the kaolin settles, re-fill the pipette. Carefully re-mix the kaolin-phospholipid mixture before each pipetting to ensure an even distribution of reagent in all tubes.
- (b) Place the kaolin-phospholipid tubes in an ice-water bath.
- (c) Using an 0.2 ml silicon-coated or plastic pipette, pipette 0.1 ml of substrate plasma into each of two kaolin-phospholipid tubes, tap gently to mix, transfer to the 37°C bath and set the interval timer to ring at 8 minutes.
- (d) After 6 minutes, prepare a 1:20 dilution of the plasma to be tested by mixing 0.1 ml of plasma with 1.9 ml barbitol-saline buffer in a cellulose-nitrate or thin-walled polypropylene tube, kept in the ice bath, using a 0.2 ml silicone pipette to transfer the plasma. Invert the tube over parafilm to mix.

(e) At 8 minutes, transfer with a plastic or silicone-coated pipette, 0.1 ml of diluted plasma to each substrate-kaolin-Gliddex tube and immediately add 0.1 ml 0.025 M calcium chloride, using 0.2 ml glass pipettes. Start the stop watches with each addition of calcium chloride. Mix immediately, and then let the tubes stand at 37°C until the watches read 30 seconds. Then mix gently and tilt the tubes continually until the clotting occurs. The tubes are tilted by rotating the wrist; they should be held one beside the other, in such a way that the degree of rotation is the same in both tubes. The end-point is usually sharp, a firm clot appearing suddenly; stop the appropriate watch as soon as a clot is noted. Record the clotting time to the nearest tenth second.

(f) If the plasma of the subject under test is more than 2 seconds different from the "control" plasma, repeat the assay with the plasma with the shorter clotting time, diluted 1:40; this should be a fresh dilution, rather than a serial dilution of the 1:20. Repeat the process until the clotting time is longer than that of the other plasma.

(g) To determine the "per cent activity" of the plasma with the longer clotting time, plot the clotting times of the plasma which has been calibrated on log-log paper and read the activity of the other by interpolation.

(h) A standard frozen pooled plasma may be used for the calibration curve. In this case, a fresh normal plasma should also be tested at a concentration of 1:20.

(6) Erythrocyte sedimentation rate (E. S. R.)

The method used is that of Westergren (1921). Venous blood is rediluted with 3.8 per cent trisodium citrate in the proportion of 1 part of citrate to 4 parts of blood. The sample is then drawn up in the Westergren tube to the 200 mm mark. The tube is placed exactly vertical and left undisturbed for 60 minutes. The height of the clear plasma above the upper limit of the column of sedimenting red cells is then read to the nearest millimetre. This figure in millimetres per hour is the E. S. R. Westergren, A. (1921) Acta medica Scandinavica, 54, 247

APPENDIX 4

ADDITIONAL CASE HISTORY

APPENDIX 4

ADDITIONAL CASE HISTORY

A case of intravascular haemolysis occurring during cephalixin therapy and associated with massive elevation of the plasma fibrinogen

We describe here the case of a haemophilic boy who developed acute intravascular haemolysis while recovering from repair of a ruptured ureter. At this time his fibrinogen level was 1000 mg/100 ml and he was taking cephalixin. The cause of the haemolysis has not been clearly defined.

Case history

The patient was a severely affected haemophiliac with no antihaemophilic factor (AHF) activity detectable in the plasma. He sustained a rupture of the ureter, multiple fractures of ribs and large haematomata of head, arms and legs as the result of a crushing injury. This was associated with intraperitoneal bleeding and a fall in haemoglobin from 15 to 6.2/100 ml. Surgery to the ureter was undertaken under cover of cryoglobulin precipitate, prepared by cold precipitation of human plasma (Pool et al., 1964). Four units of donor blood were transfused to

bring the haemoglobin to 12 G/100 ml. No serological abnormalities were detected at the time of performing the matching tests. The infusions of cryoprecipitate were continued for thirty-two days to allow complete healing of the ureter, skin incision and the drainage tube tracks. On the sixth postoperative day the patient developed haematuria and a haematoma of the thigh at the site of an intramuscular injection when the APTT activity of the plasma fell below the haemostatic level. This was the only time the patient had any evidence of bleeding during the postoperative period.

During anaesthesia the patient aspirated gastric content and developed a pneumonitis of the left lower lobe and collapse of the right upper lobe. This was treated with ampicillin, 1 G/day for nine days, then with cephalixin, 2 G/day for thirteen days.

Blood urea was elevated to 50 mg/100 ml immediately after surgery but rapidly fell to normal and at the time cephalixin was administered ranged from 20 - 25 mg/100 ml.

Nine days after the start of cephalixin therapy the patient's plasma was noted to be brown in colour and the following results suggestive of acute intravascular haemolysis were obtained;

methaemalbumin 2.34 mg/100 ml, plasma haemoglobin 6 mg/100 ml, reticulocyte count 5 per cent, total bilirubin 1.6 mg/100 ml.

Direct broad spectrum antihuman globulin (Coombs') test using a standard slide technique was negative and the osmotic fragility of the red blood cells was normal. Haemolysis increased and maximum values were seen on the thirteenth day of cephalixin therapy when the reticulocyte count was 13 per cent, the methaemalbumin 3.47 mg/100 ml and the plasma haemoglobin 8.9 mg/100 ml.

The serum complement level was normal. The maximum serum level of cephalixin obtained during this time was of 10 μ g/ml, three hours after an oral dose of 500 mg and this occurred on the twenty-third postoperative day. Serum proteins were slightly elevated, the total being 8.7 G/100 ml with an albumin of 4.6 G/100 ml (55 per cent) and globulin of 4.1 G/100 ml. Electrophoresis of plasma proteins showed elevation of the gammaglobulin fraction to 2.4 G/100 ml (25.4 per cent). β -globulin 8.5 per cent, α_1 globulin 8 per cent and α_2 globulin 3.1 per cent were within normal limits. Plasma fibrinogen was grossly elevated at 1067 mg/100 ml.

Since it is reported (Kosakai and Miyakawa, 1970) that the direct antiglobulin test on the red blood cells of patients receiving cephalosporins varies, depending upon the particular drug given and the particular antihuman globulin reagents and technique employed, it was decided to attempt to enhance the sensitivity of the method by using a two-stage method modified from that of Cohen and Nelken (1964). The patient's thrice washed red blood corpuscles, collected soon after cessation of cephalixin therapy, were incubated at room temperature with goat antihuman serum, washed to remove free serum and followed by a rabbit antigoat serum on a slide. Careful attention was given to finding the optimal dilutions for the rabbit antigoat and goat antihuman sera. The patient's cells tested by this technique gave a positive reaction, control sensitised and unsensitised cells reacting satisfactorily. No irregular blood group antibodies were detected in the patient's serum. Direct antiglobulin reactions were performed using specific goat antihuman IgG immunoglobulin and anti-B₁C component of complement with negative results. This is in agreement with Molthan et al. (1967) who reported the

coating on the cells of their patients to be non-specific protein in nature.

Cephalexin was stopped at this time and thereafter the levels of plasma haemoglobin, methaemalbumin and the reticulocyte count fell and haemoglobin level rose to normal.

Discussion

All members of the cephalosporin group of drugs are known to bind normal plasma proteins to red cells in vitro and so produce a positive direct anti-globulin test (Kosakai and Miyakawa, 1970; Mine et al., 1970). The antiglobulin reaction is dependent on the type of antiserum used and in in vitro experiments the incidence of positive tests rises with the concentration of the drug.

Molthan et al. (1967) reported positive direct Coombs' tests in 75 per cent of patients receiving cephalothin. This finding was confirmed by Gralnick et al. (1967) who showed that 40 per cent of patients on cephalothin developed a positive Coombs' test. In these series, patients with impaired renal function had

a high incidence of positive tests probably because of the high blood cephalothin concentrations and because azotaemic red cells were more easily sensitised. The incidence of positive tests was also higher in patients with hypoalbuminaemia.

A similar result was reported by York and Landes (1968) and Foord (1970) in patients receiving cephaloridine. In the latter study involving sixty-five patients, the overall incidence of positive Coombs' tests was 15 per cent but all the positive tests occurred in the group of patients receiving over 4 G of cephaloridine/day. This gave an incidence of 26 per cent in this group. Perkins et al. (1967) reported similar observations in patients receiving cephalothin or cephaloridine. Girdwood (1971) reviewing the notifications to the Safety of Drugs Committee cites eight examples.

At least five cases of positive Coombs' tests have been recorded after cephalixin therapy. Two cases are quoted by Foord (1970), one of these being in a monkey and one case by Fass et al. (1970). Also Erikssen et al. (1970) report that two

out of five patients on cephalixin developed a positive Coombs' test as determined by a 'tube' technique although the slide test was negative.

Positive Coombs' tests in patients on cephalosporins have been regarded as laboratory curiosities and of no significance with regard to haemolytic anaemia; however, two cases of acute haemolysis have been described (Kaplan et al., 1968; Foord, 1971). The first patient received cephaloridine and the second cephalixin. Both patients had, however, received penicillin therapy prior to the cephalosporin and both had impaired renal function. The incidence of cross-sensitisation reactions between penicillin and cephalosporins is probably between 10 and 15 per cent (Ky et al., 1970) but varies widely according to the criteria chosen.

The case presented here is complicated by the prior administration of ampicillin, the elevation of the gammaglobulin and the high fibrinogen level, but renal function, plasma albumin and the plasma level of cephalixin were all within normal limits. During the whole of this haemolytic episode and for ten days thereafter, the patient received cryoglobulin precipitate and had no evidence of bleeding.